DERWENT-ACC-NO: 2000-587255
DERWENT-WEEK: 200503

TITLE: Immunization formulations useful for stimulating cytokines in hosts, comprise antigens and adjuvants, especially emulsan or its analog

Basic Abstract Text (1):
NOVELTY - An immunization formulation comprising an antigen and an emulsan or emulsan analog, is new.

Basic Abstract Text (4):
Forty 6-8 week-old female BALB/c mice were randomly placed in eight groups of five mice and immunized. Pre-immune sera was obtained 3 days prior to primary immunization. An antigen (dinitrophenol coupled to keyhole limpet hemocyanin referred to as DNP-KLH) and adjuvant were mixed by repeated aspiration through an 18-gage needle. Each mouse was immunized intraperitoneally with a 200 mu l total volume of adjuvant and antigen. Mice were boosted after 28 days, and sera were taken every 3 days after boosting until day 21 post-boost, and then again at 6 weeks and 9 weeks. Total DNP-specific antibody titers was determined by ELISA (enzyme linked immunosorbant assay). Controls included injection of mice with emulsan alone in the absence of antigen. An examination of gross pathology was performed, and tissue sections from spleen, liver, lung, kidney, heart, injection site and draining lymph nodes were prepared and examined for signs of inflammation or necrosis. Results not given.

Basic Abstract Text (6):
USE - Emulsan (or analog of emulsan) are used as adjuvants with antigen for stimulating cytokines in hosts by immunomodulation of the host (which is preferably a cell line or a mammal) (claimed).

Basic Abstract Text (7):
ADVANTAGE - Unlike prior art adjuvants, the emulsan or its analog has a capacity to generate an immune response with minimal side effects and induces the production of specific antibody and T-cell response, resulting in release of cytokines. The adjuvant has improved shelf life and is more stable.

Previous Doc Next Doc Go to Doc#

Molecular Sequence Databank Number: GENBANK/S76860
CAS Registry Number: 0 (Bacterial Proteins); 0 (Lipopolysaccharides); 0 (Membrane Glycoproteins); 0 (surface array protein, bacteria)
Record Date Created: 19950426
Record Date Completed: 19950426

6/9/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10326245 PMID: 7885229
High-frequency S-layer protein variation in Campylobacter fetus revealed by sapA mutagenesis.
Blaser M J; Wang E; Tummuru M K; Washburn R; Fujimoto S; Labigne A
Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232.
Molecular microbiology (ENGLAND) Nov 1994, 14 (3) p453-62, ISSN 0950-382X--Print Journal Code: 8712028
Contract/Grant Number: R01AI24145; AI; NIAID
Publishing Model Print
Document type: In Vitro; Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib
Campylobacter fetus utilizes paracrystalline surface (S-) layer proteins that confer complement resistance and that undergo antigenic variation to facilitate persistent mucosal colonization in ungulates. C. fetus possesses multiple homologues of sapA, each of which encode full-length S-layer proteins. Disruption of sapA by a gene targeting method (insertion of kanamycin (km) resistance) caused the loss of C. fetus cells bearing full-length S-layer proteins and their replacement by cells bearing a 50 kDa truncated protein that was not exported to the cell ***surface*** . After incubation of the mutants with serum, the survival rate was approximately 2 x 10(-2). Immunoblots of survivors showed that phenotypic reversion involving high-level production of full-length (98, 127 or 149 kDa) S-layer proteins had occurred. Revertants were serum resistant but caused approximately 10-fold less bacteraemia in orally challenged mice than did the wild-type strain. Southern hybridizations of the revertants showed rearrangement of sapA homologues and retention of the km marker. These results indicate that there exists high-frequency generation of C. fetus sapA antigenic variants, and that intracellular mechanisms acting at the level of DNA reciprocal recombination play key roles in this phenomenon.
Descriptors: *Bacterial Proteins--genetics--GE; *Campylobacter fetus--genetics--GE; *Membrane Glycoproteins; Animals; Antigenic Variation ; Antigens, Bacterial--genetics--GE; Bacteremia--etiology--ET; Bacterial Proteins--immunology--IM; Bacterial Proteins--isolation and purification --IP; Blood Bactericidal Activity; Campylobacter fetus--immunology --IM; Campylobacter fetus--pathogenicity--PY; Chromosome Mapping; Complement System Proteins--metabolism--ME; Conjugation, Genetic; Escherichia coli--genetics--GE; Freeze Etching; Gene Expression; Genes, Bacterial; Genetic Vectors; Mice; Mutagenesis
CAS Registry Number: 0 (Antigens, Bacterial); 0 (Bacterial Proteins); 0 (Membrane Glycoproteins); 0 (surface array protein, bacteria); 9007-36-7 (Complement System Proteins)
Gene Symbol: sapA
Record Date Created: 19950410
Record Date Completed: 19950410

6/9/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09841321 PMID: 8238090
Role of the S-layer proteins of Campylobacter fetus in serum-resistance and antigenic variation: a model of bacterial pathogenesis.
Blaser M J
Division of Infectious Diseases, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-2605.
American journal of the medical sciences (UNITED STATES) Nov 1993, 306 (5) p325-9, ISSN 0002-9629--Print Journal Code: 0370506
Contract/Grant Number: AI-24145; AI; NIAID
Publishing Model Print
Document type: Journal Article; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.; Review
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: AIM; INDEX MEDICUS
Campylobacter fetus are microaerophilic gram-negative bacteria that are pathogens of animals and humans. These organisms possess paracrystalline surface (S-) layers, composed of acidic high molecular weight proteins. C. fetus strains possessing S-layers are resistant to C3b binding, which explains both serum and phagocytosis-resistance. C. fetus strains also can vary the subunit protein size, crystalline structure, and antigenicity of the S-layer it expresses. Therefore, its S-layer permits C. fetus to resist complement and antibodies, two of the key defenses against extracellular pathogens. C. fetus possesses several full-length genes encoding S-layer proteins with both conserved and divergent sequences, which permits gene rearrangement and antigenic variation. (24 Refs.)
Descriptors: *Antigenic Variation--physiology--PH; *Bacterial Outer Membrane Proteins--immunology--IM; *Blood Bactericidal Activity--immunology--IM; *Campylobacter fetus--pathogenicity--PY; Campylobacter fetus--immunology--IM; Conserved Sequence; Genes, Bacterial; Humans; Molecular Weight; Phagocytosis
CAS Registry Number: 0 (Bacterial Outer Membrane Proteins)
Gene Symbol: sapA
Record Date Created: 19931201
Record Date Completed: 19931201

6/9/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09747268 PMID: 7688715
Shift in S-layer protein expression responsible for antigenic variation in ***Campylobacter*** fetus.
Wang E; Garcia M M; Blake M S; Pei Z; Blaser M J
Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232.
Journal of bacteriology (UNITED STATES) Aug 1993, 175 (16) p4979-84, ISSN 0021-9193--Print Journal Code: 2985120R
Contract/Grant Number: AI24145; AI; NIAID
Publishing Model Print
Document type: Journal Article; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

Campylobacter fetus strains possess regular paracrystalline surface layers (S-layers) composed of high-molecular-weight proteins and can change the size and crystalline structure of the predominant protein expressed. Polyclonal antisera demonstrate antigenic cross-reactivity among these proteins but suggest differences in epitopes. Monoclonal antibodies to the 97-kDa S-layer protein of Campylobacter fetus subsp. fetus strain 82-40LP showed three different reactivities. Monoclonal antibody 1D1 recognized 97-kDa S-layer proteins from all C. fetus strains studied; reactivity of monoclonal antibody 6E4 was similar except for epitopes in S-layer proteins from reptile strains and strains with type B lipopolysaccharide. Monoclonal antibody 2E11 only recognized epitopes on S-layer proteins from strains with type A lipopolysaccharide regardless of size. In vitro shift from a 97-kDa S-layer protein to a 127-kDa S-layer protein resulted in different reactivity, indicating that size change was accompanied by antigenic variation. To examine in vivo variation, heifers were genetically challenged with Campylobacter fetus subsp. venerealis strains and the S-layer proteins from sequential isolates were characterized. Analysis with monoclonal antibodies showed that antigenic reactivities of the S-layer proteins were varied, indicating that these proteins represent a system for antigenic variation.

Tags: Female

Descriptors: *Antigens, Bacterial--immunology--IM; *Bacterial Proteins --immunology--IM; *Campylobacter fetus--immunology--IM; *Membrane Proteins--immunology--IM; *Variation (Genetics); Animals; Antibodies, Monoclonal; Antigens, Bacterial--metabolism--ME; Bacterial Proteins --metabolism--ME; Campylobacter Infections--immunology--IM; Campylobacter Infections--veterinary--VE; Campylobacter fetus --metabolism--ME; Cattle; Cross Reactions; Epitopes; Membrane Proteins --metabolism--ME; Peptide Fragments--immunology--IM; Serine Endopeptidases --metabolism--ME; Species Specificity; Uterus--microbiology--MI

CAS Registry Number: 0 (Antibodies, Monoclonal); 0 (Antigens, Bacterial); 0 (Bacterial Proteins); 0 (Epitopes); 0 (Membrane Proteins); 0 (Peptide Fragments)

Enzyme Number: EC 3.4.21.- (Serine Endopeptidases); EC 3.4.21.19 (glutamyl endopeptidase)

Record Date Created: 19930910

Record Date Completed: 19930910

6/9/18 (Item 18 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

09743199 PMID: 8346244

Rearrangement of sapA homologs with conserved and variable regions in ***Campylobacter*** fetus.

Tummuru M K; Blaser M J

Vanderbilt University School of Medicine, Division of Infectious Diseases, Nashville, TN 37232-2605.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Aug 1 1993, 90 (15) p7265-9, ISSN 0027-8424--Print Journal Code: 7505876

Contract/Grant Number: R01A1-24145; PHS

Publishing Model Print

Document type: Comparative Study; Journal Article; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

The Campylobacter fetus surface -layer (S-layer) proteins mediate both complement resistance and antigenic variation in mammalian hosts. Wild-type strain 23D possesses the sapA gene, which encodes a 97-kDa S-layer protein, and several sapA homologs are present in both wild-type

Mice; Phagocytosis; Pronase--metabolism--ME; Virulence
CAS Registry Number: 0 (Antigens, Bacterial); 0 (Bacterial Proteins); 0 (Membrane Glycoproteins); 0 (surface array protein, bacteria)
Enzyme Number: EC 3.4.24.- (Pronase)
Record Date Created: 19930212
Record Date Completed: 19930212

6/9/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09377737 PMID: 1522068
Characterization of the Campylobacter fetus sapA promoter: evidence that the sapA promoter is deleted in spontaneous mutant strains.
Tummuru M K; Blaser M J
Division of Infectious Diseases, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-2605.
Journal of bacteriology (UNITED STATES) Sep 1992, 174 (18) p5916-22, ISSN 0021-9193--Print Journal Code: 2985120R
Contract/Grant Number: R01 AI24145; AI; NIAID
Publishing Model Print
Document type: Journal Article; Research Support, U.S. Gov't, P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib
Wild-type Campylobacter fetus cells possess S-layer proteins (S+ phenotype), whereas after laboratory passage, spontaneous stable mutants that do not express these proteins (S- phenotype) arise. To determine the molecular mechanisms by which C. fetus changes to the S- phenotype, we studied wild-type strain 23D, from which the sapA gene encoding the 97-kDa S-layer protein has been cloned, and strain 23B, a spontaneous S- mutant. We compared these strains with another pair of strains, LP (S+) and HP (S-). Southern analysis with the cloned sapA gene as a probe indicated that both pairs of strains have multiple sapA homologs. Using gene disruption and replacement techniques, we constructed an isogenic strain of 23D that differed only in sapA expression (strain 23D:401:1). A 6.0-kb HindIII fragment from 23D:401:1 containing 3.4 kb of sapA upstream region then was cloned into pBluescript to produce pBG101. Nucleotide sequence analysis of sapA upstream region revealed a consensus promoter at -121 bp from the translational start site. Primer extension analysis placed a single in vivo transcription initiation site at the -114-bp position of sapA. A DNA probe derived from the sapA promoter region hybridized to a 5.5-kb HindIII fragment of chromosomal DNA from strain 23D but not to DNA from strain 23B. Northern RNA blot analysis showed no sapA mRNA in strain 23B. These data indicate that the lack of S-layer protein expression in spontaneous mutant strains is caused by the deletion of promoter sequences.
Descriptors: *Antigens, Bacterial--genetics--GE; *Bacterial Proteins --genetics--GE; *Campylobacter fetus--genetics--GE; *Membrane Glycoproteins; *Promoter Regions (Genetics)--genetics--GE; Bacterial Proteins--biosynthesis--BI; Base Sequence; Blotting, Southern; Chromosome Mapping; Cloning, Molecular; Gene Expression Regulation, Bacterial; Molecular Sequence Data; Multigene Family--genetics--GE; Mutagenesis --genetics--GE; Phenotype; Polymerase Chain Reaction; Variation (Genetics)
Molecular Sequence Databank Number: GENBANK/M93985; GENBANK/M94060; GENBANK/M94061; GENBANK/M94062; GENBANK/M94629; GENBANK/S44580; GENBANK/X64378; GENBANK/X64379; GENBANK/X64380; GENBANK/X64381
CAS Registry Number: 0 (Antigens, Bacterial); 0 (Bacterial Proteins); 0 (Membrane Glycoproteins); 0 (surface array protein, bacteria)
Gene Symbol: SapA
Record Date Created: 19921013
Record Date Completed: 19921013

6/9/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09176967 PMID: 1735716
Reattachment of surface array proteins to Campylobacter fetus cells.
Yang L Y; Pei Z H; Fujimoto S; Blaser M J
Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232.
Journal of bacteriology (UNITED STATES) Feb 1992, 174 (4) p1258-67, ISSN 0021-9193--Print Journal Code: 2985120R
Contract/Grant Number: R01AI-24145; AI; NIAID
Publishing Model Print
Document type: Journal Article; Research Support, U.S. Gov't, P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Campylobacter fetus strains may be of serotype A or B, a property associated with lipopolysaccharide (LPS) structure. Wild-type C. fetus strains contain surface array proteins (S-layer proteins) that may be extracted in water and that are critical for virulence. To explore the relationship of S-layer proteins to other surface components, we reattached S-layer proteins onto S- template cells generated by spontaneous mutation or by serial extractions of S+ cells with water. Reattachment occurred in the presence of divalent (Ba2+, Ca2+, Co2+, and Mg2+) but not monovalent (H+, NH4+, Na+, K+) or trivalent (Fe3+) cations. The 98-, 125-, 127-, and 149-kDa S-layer proteins isolated from strains containing type A LPS (type A S-layer protein) all reattached to S- template cells containing type A LPS (type A cells) but not to type B cells. The 98-kDa type B S-layer protein reattached to SAP- type B cells but not to type A cells. Recombinant 98-kDa type A S-layer protein and its truncated amino-terminal 65- and 50-kDa segments expressed in Escherichia coli retained the full and specific determinants for attachment. S-layer protein and purified homologous but not heterologous LPS in the presence of calcium produced insoluble complexes. By quantitative enzyme-linked immunosorbent assay, the S-layer protein copy number per C. fetus cell was determined to be approximately 10(5). In conclusion, C. fetus cells are encapsulated by a large number of S-layer protein molecules which may be specifically attached through the N-terminal half of the molecule to LPS in the presence of divalent cations.
Descriptors: *Bacterial Capsules--metabolism--ME; *Bacterial Outer Membrane Proteins--metabolism--ME; *Bacterial Proteins; *Campylobacter fetus--metabolism--ME; *Lipopolysaccharides--metabolism--ME; *Membrane Glycoproteins--metabolism--ME; Animals; Bacterial Capsules--chemistry--CH; Bacterial Capsules--ultrastructure--UL; Bacterial Outer Membrane Proteins--chemistry--CH; Bacterial Outer Membrane Proteins--ultrastructure--UL; Campylobacter fetus--chemistry--CH; Campylobacter fetus--ultrastructure--UL; Cations--metabolism--ME; Enzyme-Linked Immunosorbent Assay; Humans; Isoelectric Point; Membrane Glycoproteins--chemistry--CH; Membrane Glycoproteins--ultrastructure--UL; Microscopy, Electron
CAS Registry Number: 0 (Bacterial Capsules); 0 (Bacterial Outer Membrane Proteins); 0 (Bacterial Proteins); 0 (Cations); 0 (Lipopolysaccharides); 0 (Membrane Glycoproteins); 0 (surface array protein, bacteria)
Record Date Created: 19920310
Record Date Completed: 19920310

6/9/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08963679 PMID: 1885571
Identification, purification, and characterization of major antigenic proteins of ***Campylobacter*** jejuni.
Pei Z H; Ellison R T; Blaser M J
Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232.
Journal of biological chemistry (UNITED STATES) Sep 5 1991, 266 (25) p16363-9, ISSN 0021-9258--Print Journal Code: 2985121R
Publishing Model Print
Document type: Journal Article; Research Support, U.S. Gov't, Non-P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Evidence from developing countries and volunteer studies indicates that immunity to Campylobacter jejuni and Campylobacter coli may be acquired, but the antigenic basis for this protection is poorly defined. We have purified to homogeneity four proteins with molecular weights of 28,000 (PEB1), 29,000 (PEB2), 30,000 (PEB3), and 31,000 (PEB4) from epidemic C. jejuni strain 81-176 using acid extraction and sequential ion-exchange, hydrophobic interaction, and gel filtration chromatography. The relative amino acid compositions of these four proteins are similar. NH2-terminal sequence analysis indicates that all four proteins are different, although the first 35 amino acids of PEB2 and ***PEB3*** are 51.4% homologous. Isoelectric focusing showed that all four are basic proteins with pI of 8.5 for PEB1 protein and greater than 9.3 for the others. Use of the purified proteins as antigens in an IgG enzyme-linked immunosorbent assay (ELISA) found that seroconversion to the PEB1 or PEB3 proteins occurred in 15 of 19 patients with sporadic C. jejuni or C. coli infection. In comparison, only two, six, and 14 of these patients seroconverted to PEB2, PEB4, or the acid extract antigen. In an ELISA with whole bacterial cells as antigens, antiserum to the acid-extracted antigens showed broad recognition of C. jejuni, C. coli, C. fetus, C. lari, and Helicobacter pylori. Antiserum to PEB1 recognized all 35 C. jejuni and all 15 C. coli strains but none of the isolates of the other three bacterial species. The PEB1 and ***PEB3*** proteins appear to be candidate antigens for both a Campylobacter vaccine and for serological assays for the pathogen.
Descriptors: *Antigens, Bacterial--isolation and purification--IP; *Bacterial Proteins--isolation and purification--IP; *Campylobacter jejuni--immunology--IM; Amino Acid Sequence; Amino Acids--analysis--AN; Animals; Antigens, Bacterial--chemistry--CH; Bacterial Proteins--chemistry --CH; Blotting, Western; Campylobacter Infections--microbiology--MI; Campylobacter jejuni--isolation and purification--IP; Chromatography, High Pressure Liquid; Diarrhea--microbiology--MI; Electrophoresis, Polyacrylamide Gel; Endopeptidases; Enzyme-Linked Immunosorbent Assay; Helicobacter pylori--isolation and purification--IP; Humans; Isoelectric Point; Molecular Sequence Data; Molecular Weight; Primates
CAS Registry Number: 0 (Amino Acids); 0 (Antigens, Bacterial); 0 (Bacterial Proteins)
Enzyme Number: EC 3.4.- (Endopeptidases)
Record Date Created: 19911004
Record Date Completed: 19911004

6/9/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08864543 PMID: 2037362
Correlation between molecular size of the surface array protein and morphology and antigenicity of the ***Campylobacter*** fetus S layer.
Fujimoto S; Takade A; Amako K; Blaser M J

Department of Bacteriology, Faculty of Medicine, Kyushu University, Fukuoka, Japan.
Infection and immunity (UNITED STATES) Jun 1991, 59 (6) p2017-22, ISSN 0019-9567--Print Journal Code: 0246127
Contract/Grant Number: AI 24145; AI; NIAID
Publishing Model Print
Document type: Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

The correlation between the molecular size of the surface layer protein (S protein) and both structure and antigenicity of the Campylobacter fetus surface layer (S layer) was investigated in several clinical strains and their spontaneous variants which produce S proteins of molecular weights (MW) different from those of the parents. Only three molecular sizes of the S proteins were observed (98, 127, and 149 kDa) in the parental and variant strains. Immunologically, the 98-kDa protein and the 149-kDa protein but not the 127-kDa protein were cross-reactive. Freeze-etching analysis showed that the 98-kDa S protein formed a hexagonal arrangement with a 24-nm center-to-center space and that the S proteins with larger MW (127 or 149 kDa) formed tetragonal ones with an 8-nm center-to-center space. Thus, the MW changes of the S proteins seen in the variant strains were associated with both morphological and antigenic changes in S layer. These observations support the hypothesis that the pattern and antigenicity of the C. fetus S layer is determined by the particular type of S protein. Furthermore, the presence of the two different S layer patterns on a single bacterial cell indicates that multiple S proteins can be produced and expressed in a single cell.

Tags: Male
Descriptors: *Antigens, Bacterial--immunology--IM; *Bacterial Outer Membrane Proteins--chemistry--CH; *Bacterial Proteins; *Campylobacter fetus--analysis--AN; *Membrane Glycoproteins--chemistry--CH; Animals; Bacterial Outer Membrane Proteins--immunology--IM; Bacterial Outer Membrane Proteins--ultrastructure--UL; Campylobacter fetus --immunology--IM; Campylobacter fetus--ultrastructure--UL; Cross Reactions--immunology--IM; Electrophoresis, Polyacrylamide Gel; Freeze Fracturing; Image Processing, Computer-Assisted; Immunoblotting; Membrane Glycoproteins--immunology--IM; Membrane Glycoproteins--ultrastructure--UL; Mice; Molecular Weight
CAS Registry Number: 0 (Antigens, Bacterial); 0 (Bacterial Outer Membrane Proteins); 0 (Bacterial Proteins); 0 (Membrane Glycoproteins); 0 (surface array protein, bacteria)
Record Date Created: 19910628
Record Date Completed: 19910628

6/9/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08758841 PMID: 2097496
Adherence of Helicobacter pylori cells and their surface components to HeLa cell membranes.
Fauchere J L; Blaser M J
Research Service, Veterans Administration Medical Center, Denver, Colorado.
Microbial pathogenesis (ENGLAND) Dec 1990, 9 (6) p427-39, ISSN 0882-4010--Print Journal Code: 8606191
Publishing Model Print
Document type: Journal Article; Research Support, Non-U.S. Gov't
Languages: ENGLISH

Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

Four Helicobacter pylori strains were used to develop in vitro methods to assess adherence to HeLa cells. Using direct detection by microscopy, adhesion scores increased with the initial bacteria-to-cell ratio. The urease method assessed H. pylori bound to HeLa cells by their urease activity. The percentage of the original inoculum adhering to HeLa cells remained constant for initial ratios from 10(2) to 10(5) bacteria per cell. An ELISA using anti-H. pylori serum assessed whole bacteria or components bound to HeLa cell fractions. By all three methods, the four H. pylori strains were adherent to HeLa cells or membranes whereas ***Campylobacter*** fetus and Providencia control strains were not. The adherence of H. pylori whole cells decreased following extraction with saline, water, or glycine buffer and most of the superficial adhering material (SAM) was present in the saline or water extracts. SAM bound better to HeLa membranes than to calf fetuin or bovine serum albumin (BSA); binding was inhibited by preincubation of SAM with HeLa membranes but not with fetuin or BSA or by pretreatment of HeLa membranes with neuraminidase. These data indicate that SAM has a specific receptor on the HeLa cell membranes. By gel exclusion chromatography of bacterial extracts, the most adherent components were found in the fractions which also contained the highest urease activity; these fractions included urease subunit antigens. We conclude that adherence of H. pylori can be assessed by microtiter assays and involves bacterial surface material which co-purifies with urease and is different from the N-acetyl-neuraminyl-lactose binding hemagglutinin.

Descriptors: *Bacterial Adhesion; *Helicobacter pylori--physiology--PH; Bacterial Proteins--analysis--AN; Blotting, Western; Cell Adhesion Molecules--analysis--AN; Cell Membrane--microbiology--MI; Chromatography, Gel; Enzyme-Linked Immunosorbent Assay; Hela Cells; Helicobacter pylori --enzymology--EN; Helicobacter pylori--ultrastructure--UL; Humans; Urease --metabolism--ME

CAS Registry Number: 0 (Bacterial Proteins); 0 (Cell Adhesion Molecules)
Enzyme Number: EC 3.5.1.5 (Urease)
Record Date Created: 19910724
Record Date Completed: 19910724

6/9/26 (Item 26 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08634779 PMID: 2229082

Surface array protein of ***Campylobacter*** fetus. Cloning and gene structure.

Blaser M J; Gotschlich E C

Journal of biological chemistry (UNITED STATES) Nov 5 1990, 265 (31) p19372, ISSN 0021-9258--Print Journal Code: 2985121R
Publishing Model Print; Erratum for J Biol Chemical 1990 Aug 25;265(24) 14529-35; Erratum for PMID 2387868
Document type: Published Erratum
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

Descriptors: *Bacterial Outer Membrane Proteins--genetics--GE; *Bacterial Proteins; *Campylobacter fetus--genetics--GE; *Genes, Bacterial; *Membrane Glycoproteins--genetics--GE; Amino Acid Sequence; Base Sequence; Molecular Sequence Data

Molecular Sequence Databank Number: GENBANK/J05577

CAS Registry Number: 0 (Bacterial Outer Membrane Proteins); 0 (Bacterial Proteins); 0 (Membrane Glycoproteins); 0 (surface array protein, bacteria)

Record Date Created: 19901210
Record Date Completed: 19901210

6/9/27 (Item 27 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08572288 PMID: 2387622
Surface array proteins of Campylobacter fetus block lectin-mediated binding to type A lipopolysaccharide.
Fogg G C; Yang L Y; Wang E; Blaser M J
Medical Service, Veterans Administration Medical Center, Denver, Colorado 80220.
Infection and immunity (UNITED STATES) Sep 1990, 58 (9) p2738-44, ISSN 0019-9567--Print Journal Code: 0246127
Contract/Grant Number: R01 AI24145; AI; NIAID
Publishing Model Print
Document type: Journal Article; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Campylobacter fetus strains with type A lipopolysaccharide (LPS) and a surface array protein layer (S+) have been found to be pathogenic in humans and animals. Spontaneous laboratory mutants that lack surface array proteins (S-) are sensitive to the bactericidal activity of normal human serum. The ability of lectins to determine the presence of the S-layer and differentiate LPS type was assessed. We screened 14 lectins and found 3 (wheat germ agglutinin, Bandeiraea simplicifolia II, and Helix pomatia agglutinin) that agglutinated S- C. fetus strains with type A LPS but not S- strains with type B or type C LPS or S+ strains. However, the S+ type A strains were agglutinated after sequential water extraction, heat, or pronase treatment, all of which remove the S-layer, whereas there was no effect on the control strains. Specific carbohydrates for each lectin and purified LPS from a type A C. fetus strain specifically inhibited agglutination of an S- type A strain. In a direct enzyme-linked lectin assay, binding to the S- type A LPS strain was significantly greater than binding to the S+ strain (P = 0.01) or to a ***Campylobacter*** jejuni strain (P = 0.008). Consequently, these results indicate that the three lectins bind to the O side chains of C. fetus type A LPS but that the presence of the S-layer on intact cells blocks binding.
Descriptors: *Campylobacter fetus--classification--CL; *Lectins --metabolism--ME; *Lipopolysaccharides--pharmacology--PD; *Membrane Proteins--pharmacology--PD; *Receptors, Mitogen--metabolism--ME; Agglutination Tests; Campylobacter fetus--drug effects--DE; Campylobacter fetus--metabolism--ME; Endopeptidase K; Receptors, Mitogen--drug effects--DE; Serine Endopeptidases--pharmacology--PD; Sugar Acids--pharmacology--PD
CAS Registry Number: 0 (Lectins); 0 (Lipopolysaccharides); 0 (Membrane Proteins); 0 (Receptors, Mitogen); 0 (Sugar Acids); 1069-03-0 (2-keto-3-deoxyoctonate)
Enzyme Number: EC 3.4.21.- (Serine Endopeptidases); EC 3.4.21.64 (Endopeptidase K)
Record Date Created: 19900927
Record Date Completed: 19900927

6/9/28 (Item 28 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08565093 PMID: 2384045

Helicobacter pylori-related gastroduodenal disease in children. Diagnostic utility of enzyme-linked immunosorbent assay.
Glassman M S; Dallal S; Berezin S H; Bostwick H E; Newman L J; Perez-Perez G I; Blaser M J
Division of Pediatric Gastroenterology, New York Medical College, Valhalla 10595.
Digestive diseases and sciences (UNITED STATES) Aug 1990, 35 (8) p993-7, ISSN 0163-2116--Print Journal Code: 7902782
Publishing Model Print
Document type: Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: AIM; INDEX MEDICUS

To evaluate the accuracy of IgG and IgA serological tests in establishing a diagnosis of Helicobacter (Campylobacter) pylori gastric infection, 60 children presenting with chronic abdominal pain were prospectively studied. Endoscopic antral biopsies were obtained and analyzed for the presence of H. pylori using three standard methods: culture and identification of bacterial isolates, microscopic examination for morphologically characteristic bacteria, and urease production by the biopsy specimen. Concomitantly obtained serum samples were analyzed for the presence of IgG and IgA antibodies against H. pylori ***surface*** antigens using enzyme-linked immunosorbent assay (ELISA). Thirty-four of 60 (56.6%) had histological evidence of chronic active gastritis, eight of whom (13.3%) also had evidence of H. pylori infection by at least one criteria. Six of the eight infected patients had H. pylori demonstrated by all three methods. Of the eight infected patients, seven had IgG antibodies against H. pylori (sensitivity of 87%) and six had IgA antibodies (sensitivity of 75%). Among the six patients who had H. pylori infection confirmed by all three methods, all had IgG antibodies (sensitivity of 100%). In the patients without evidence of H. pylori infection, the IgG ELISA had a specificity of 96% (50/52), and the IgA ELISA had a specificity of 100% (52/52). Our data suggest that serological testing for the presence of antibodies against H. pylori may be a useful diagnostic tool in screening children with chronic abdominal pain for the presence of gastric infection with H. pylori.

Tags: Female; Male
Descriptors: *Campylobacter Infections--diagnosis--DI; *Duodenitis --diagnosis--DI; *Gastritis--diagnosis--DI; Adolescent; Antibodies, Bacterial--blood--BL; Biopsy; Campylobacter--immunology--IM; Campylobacter--isolation and purification--IP; Campylobacter Infections--microbiology--MI; Campylobacter Infections--pathology --PA; Child; Child, Preschool; Chronic Disease; Duodenitis--microbiology --MI; Duodenitis--pathology--PA; Duodenoscopy; Enzyme-Linked Immunosorbent Assay; Gastric Mucosa--microbiology--MI; Gastric Mucosa--pathology--PA; Gastritis--microbiology--MI; Gastritis--pathology--PA; Gastroscopy; Humans ; Immunoglobulin A--analysis--AN; Immunoglobulin G--analysis--AN; Prospective Studies
CAS Registry Number: 0 (Antibodies, Bacterial); 0 (Immunoglobulin A); 0 (Immunoglobulin G)
Record Date Created: 19900917
Record Date Completed: 19900917

6/9/29 (Item 29 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08443377 PMID: 2318963
Pathogenesis of ***Campylobacter*** fetus infections. Role of ***surface*** array proteins in virulence in a mouse model.
Pei Z; Blaser M J

Medical Service, Veterans Administration Medical Center, Denver, Colorado.
Journal of clinical investigation (UNITED STATES) Apr 1990, 85 (4) p1036-43, ISSN 0021-9738--Print Journal Code: 7802877
Contract/Grant Number: R01 AI-24145-01A1; AI; NIAID
Publishing Model Print
Document type: Journal Article; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: AIM; INDEX MEDICUS; Toxbib
We developed a mouse model to compare the virulence of Campylobacter fetus strains with (S-plus) and without (S-minus) ***surface*** array protein (S-protein) capsules. In adult HA/ICR mice pretreated with ferric chloride, the LD50 for S-plus strain 84-32 was 43.3 times lower than its spontaneous S-minus mutant 84-54. Seven strains of inbred mice were no more susceptible than the outbred strain. In contrast to the findings with Salmonella typhimurium by others, 3 X 10(7) CFU of strain 84-32 caused 90% mortality in C3H/HeN (LPSn) mice and 40% mortality in C3H/HeJ (LPSd) mice. High-grade bacteremia in HA/ICR mice occurred after oral challenge with S-plus C. fetus strains and continued for at least 2 d, but was not present in any mice challenged with S-minus strains. Bacteremia at 30 min after challenge was 51.6-fold lower in mice pretreated with 10 microliters of rabbit antiserum to purified S-protein than after pretreatment with normal rabbit serum. Challenge of mice with a mixture of S-minus strain 84-54 and free S-proteins at a concentration 31.1-fold higher than found in wild-type strain 84-32 caused 30% mortality, compared with 0% with strain 84-54 or S-protein alone. These findings in a mouse model point toward the central role of the S-protein in the pathogenesis of C. fetus infection. The S-protein is not toxic per se, but enhances virulence when present on the bacterial cell ***surface*** as a capsule.
Tags: Female
Descriptors: *Bacterial Proteins--toxicity--TO; *Campylobacter fetus--pathogenicity--PY; Animals; Bacterial Proteins--analysis--AN; Iron --metabolism--ME; Lethal Dose 50; Lipopolysaccharides--toxicity--TO; Mice; Mice, Inbred C3H; Mice, Inbred ICR; Sepsis--prevention and control--PC; Species Specificity; Virulence
CAS Registry Number: 0 (Bacterial Proteins); 0 (Lipopolysaccharides); 7439-89-6 (Iron)
Record Date Created: 19900509
Record Date Completed: 19900509

6/9/30 (Item 30 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08172870 PMID: 2760498
Clinical and immunologic significance of cholera-like toxin and cytotoxin production by Campylobacter species in patients with acute inflammatory diarrhea in the USA.
Perez-Perez G I; Cohn D L; Guerrant R L; Patton C M; Reller L B; Blaser M J
Infectious Disease Section, Veterans Administration Medical Center, Denver, CO 80220.
Journal of infectious diseases (UNITED STATES) Sep 1989, 160 (3) p460-8, ISSN 0022-1899--Print Journal Code: 0413675
Publishing Model Print
Document type: Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed

Subfile: AIM; INDEX MEDICUS; Toxbib
The humoral immune response to both Campylobacter jejuni cell surface antigens and to potential toxins of the organism was studied in 64 adults with inflammatory diarrhea. In an enzyme-linked immunosorbent assay (ELISA) for surface antigens, 17 (71%) of 24 persons with Campylobacter enteritis showed seroconversion in more than one immunoglobulin class, versus only 2 (5%) of 40 patients with non-***Campylobacter*** enteritis. In a GM1 ganglioside-based ELISA for detecting serum IgG to cholera-like enterotoxin, only one patient studied showed seroconversion to the enterotoxin. Of 22 ***Campylobacter*** isolates studied for production of cholera-like toxin, none of the supernatants from the ***Campylobacter*** strains were positive. Supernatants were also tested for enterotoxin and cytotoxic activity on Chinese hamster ovary cells; all isolates were negative for enterotoxin activity. In contrast, cytotoxin was produced by 7 (32%) isolates but was usually low-level and was not neutralized by patient's serum. These findings indicate that production of cholera-like toxin and cytotoxin by Campylobacter strains in the United States occurs in few strains and that host immune response is absent; their biologic significance in the pathogenesis of ***Campylobacter*** infections remains unclear.
Descriptors: *Campylobacter--pathogenicity--PY; *Campylobacter%% % Infections--immunology--IM; *Cholera Toxin; *Cytotoxins--biosynthesis--BI ; *Diarrhea--microbiology--MI; Acute Disease; Antibody Formation; Antigens, Surface--analysis--AN; Campylobacter--immunology--IM; Diarrhea --immunology--IM; Enterotoxins--analysis--AN; Enzyme-Linked Immunosorbent Assay; Humans; Inflammation; United States
*** CAS Registry Number: 0 (Antigens, Surface); 0 (Cytotoxins); 0*** (Enterotoxins); 9012-63-9 (Cholera Toxin)
Record Date Created: 19890919
Record Date Completed: 19890919

6/9/31 (Item 31 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08098195 PMID: 2722241
Two-dimensional gel electrophoresis and immunoblotting of ***Campylobacter*** pylori proteins.
Dunn B E; Perez-Perez G I; Blaser M J
Laboratory Service, Denver Veterans Administration Medical Center, Colorado 80220.
Infection and immunity (UNITED STATES) Jun 1989, 57 (6) p1825-33, ISSN 0019-9567--Print Journal Code: 0246127
Contract/Grant Number: BRSG-05357; RS; DRS
Publishing Model Print
Document type: Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Whole-cell, outer-membrane protein, flagellum-associated antigens and partially purified urease of Campylobacter pylori were analyzed by two-dimensional gel electrophoresis. C. pylori strains were readily distinguished from strains of ***Campylobacter*** jejuni, C. coli, and C. fetus by absence of major outer membrane proteins with Mrs of 41,000 to 45,000. C. pylori strains also lacked the acidic ***surface*** -array proteins at Mr 100,000 to 149,000 identified previously in serum-resistant strains of C. fetus. ***Surface*** labeling of intact C. pylori cells with 125I revealed two common major proteins, which we have designated protein 2 (pI 5.6 to 5.8, Mr 66,000) and protein 3 (pI 5.2 to 5.5, Mr 63,000).

Proteins 2 and 3 were also the major components (subunits) observed in partially purified urease. Partially purified preparations of flagella consistently contained proteins 2 and 3. Thus, urease appears to be associated with both outer membranes and flagella of C. pylori. C. pylori strains also possessed an antigen at Mr 59,000 which was cross-reactive with antiserum against flagella of C. jejuni. However, the antigen did not appear to be associated with flagella per se in C. pylori. Protein 2 was unique to C. pylori among the ***Campylobacter*** species studied. It was not recognized by antibody against whole cells of C. jejuni or C. fetus or flagella of C. jejuni. Protein 3 was cross-reactive with antiserum against whole cells of C. jejuni and C. fetus, as were several other major protein antigens. Because protein 2 is a major outer membrane protein that is apparently unique to C. pylori, development of monospecific antibodies against this antigen may be useful for the identification of C. pylori in tissues, and purified antigen may be useful for serologic tests for specific diagnosis of C. pylori infections.

Descriptors: *Bacterial Proteins--isolation and purification--IP; *Campylobacter--analysis--AN; *Electrophoresis, Gel, Two-Dimensional; *Immunoblotting; Animals; Antigens, Bacterial--isolation and purification --IP; Autoradiography; Flagella--analysis--AN; Flagella--immunology--IM; Humans; Immune Sera; Rabbits; Urease--immunology--IM; Urease--isolation and purification--IP

CAS Registry Number: 0 (Antigens, Bacterial); 0 (Bacterial Proteins); 0 (Immune Sera)

Enzyme Number: EC 3.5.1.5 (Urease)

Record Date Created: 19890623

Record Date Completed: 19890623

6/9/32 (Item 32 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

07750090 PMID: 3384911

Influence of strain characteristics and immunity on the epidemiology of ***Campylobacter*** infections in Thailand.

Taylor D N; Echeverria P; Pitarangsi C; Seriwatana J; Bodhidatta L; Blaser M J

Department of Bacteriology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

Journal of clinical microbiology (UNITED STATES) May 1988, 26 (5) p863-8, ISSN 0095-1137--Print Journal Code: 7505564

Publishing Model Print

Document type: Comparative Study; Journal Article; Research Support, U.S. Gov't, Non-P.H.S.

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

To determine how strain differences and immunity affect the clinical expression of Campylobacter infections, we conducted a study of acute diarrheal disease in Thailand in which specimens from children with Campylobacter infections were cultured weekly for up to 12 weeks to determine the serotype-specific length of time of convalescent-phase excretion and rate of reinfection. Levels of immunoglobulin G to cell-***surface*** antigens of C. jejuni were determined in another population of healthy children who were closely related by age and location to the children in the diarrheal disease study. ***Campylobacter*** species were initially isolated from 18% of 586 children under 5 years old with diarrhea; most isolates in Thailand belonged to serotypes commonly found in developed countries. C. coli was significantly less often associated with symptomatic infections and with bloody diarrhea than C. jejuni (P less than 0.001 and P = 0.045, respectively). The peak age of isolation and the peak level of immunoglobulin G to Campylobacter species occurred before 2

years of age. The mean duration of convalescent-phase excretion was 14 +/- 2 (standard error of the mean) days for children less than 1 year old and 8 +/- 2 days for children 1 to 5 years old (P = 0.02, t test). Infection with another Campylobacter serotype was found in 34% of 105 children during the 12-week follow-up period. The rate of reinfection in these children was 15% (range, 8 to 22%) each week. Hyperendemic exposure to Campylobacter species in Thailand confers immunity to infection that is associated with an early peak in specific serum antibodies and an age-related decrease in the case-to-infection ratio and duration of convalescent-phase excretion but does not prevent asymptomatic infections.

Descriptors: *Campylobacter--classification--CL; *Campylobacter%%% Infections--epidemiology--EP; *Diarrhea--epidemiology--EP; Age Factors; Campylobacter--immunology--IM; Campylobacter --isolation and purification--IP; Campylobacter Infections--immunology--IM; Campylobacter Infections--microbiology--MI; Child, Preschool; Developing Countries; Diarrhea--immunology--IM; Diarrhea--microbiology--MI; Humans; Immunity, Active; Infant; Serotyping; Thailand

Record Date Created: 19880801

Record Date Completed: 19880801

6/9/33 (Item 33 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

07713769 PMID: 3366901

Pathogenesis of ***Campylobacter*** fetus infections. Failure of encapsulated Campylobacter fetus to bind C3b explains serum and phagocytosis resistance.

Blaser M J; Smith P F; Repine J E; Joiner K A

Infectious Disease Section, Veterans Administration Medical Center, Denver, Colorado 80220.

Journal of clinical investigation (UNITED STATES) May 1988, 81 (5) p1434-44, ISSN 0021-9738--Print Journal Code: 7802877

Publishing Model Print

Document type: Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: AIM; INDEX MEDICUS

Campylobacter fetus ssp. fetus strains causing systemic infections in humans are highly resistant to normal and immune serum, which is due to the presence of high molecular weight (100,000, 127,000, or 149,000) ***surface*** (S-layer) proteins. Using serum-resistant parental strains (82-40 LP and 23D) containing the 100,000-mol wt protein and serum-sensitive mutants (82-40 HP and 23B) differing only in that they lack the 100,000-mol wt protein capsule, we examined complement binding and activation, and opsono-phagocytosis by polymorphonuclear leukocytes. C3 consumption was similar for all four strains but C3 was not efficiently bound to 82-40 LP or 23D even in the presence of immune serum, and the small amount of C3 bound was predominently the hemolytically inactive iC3b fragment. Consumption and binding of C5 and C9 was significantly greater for the unencapsulated than the encapsulated strains. Opsonization of 82-40 HP with heat-inactivated normal human serum caused greater than 99% killing by human PMN. Similar opsonization of 82-40 LP showed no kill, but use of immune serum restored killing. Findings in a PMN chemiluminescence assay showed parallel results. Association of 32P-labeled 82-40 HP with PMN in the presence of HINHS was 19-fold that for the 82-40 LP, and electron microscopy illustrated that the difference was in uptake rather than in binding. These results indicate that presence of the 100,000-mol wt protein capsule on the ***surface*** of C. fetus leads to impaired C3b binding, thus explaining serum resistance and defective opsonization in NHS, mechanisms that explain the capacity of this enteric organism to cause

systemic infections.
Descriptors: *Campylobacter Infections--etiology--ET; * Campylobacter fetus--immunology--IM; *Complement C3--metabolism--ME; *Neutrophils--immunology--IM; Blood Bactericidal Activity; Campylobacter Infections--immunology--IM; Campylobacter fetus--pathogenicity --PY; Campylobacter fetus--ultrastructure--UL; Complement C3 --analysis--AN; Complement C3--immunology--IM; Complement C5--immunology --IM; Complement C5--metabolism--ME; Complement C9--immunology--IM; Complement C9--metabolism--ME; Humans; Microscopy, Electron; Opsonin Proteins; Phagocytosis; Virulence
CAS Registry Number: 0 (Complement C3); 0 (Complement C5); 0 (Complement C9); 0 (Opsonin Proteins)
Record Date Created: 19880614
Record Date Completed: 19880614

6/9/34 (Item 34 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07701557 PMID: 3360785
Purification and characterization of a family of high molecular weight ***surface*** -array proteins from ***Campylobacter*** fetus.
Pei Z; Ellison R T; Lewis R V; Blaser M J
Medical Service, Veterans Administration Medical Center, Denver, Colorado.
Journal of biological chemistry (UNITED STATES) May 5 1988, 263 (13) p6416-20, ISSN 0021-9258--Print Journal Code: 2985121R
Contract/Grant Number: BRSG-0537; RS; DRS; RR02035; RR; NCRR
Publishing Model Print
Document type: Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
A variety of Gram-negative and Gram-positive bacteria possess crystalline ***surface*** layers, although little is known of their function. We previously have shown that the high molecular weight surface-array proteins of Campylobacter fetus are important in both the pathogenicity and antigenicity of this organism. For biochemical and immunological characterization, we purified high molecular weight (100,000, 127,000, 149,000) ***surface*** -array proteins from three C. fetus strains using sequential gel filtration and ion exchange high performance liquid chromatography. These proteins are acidic with pI values between 4.12 and 4.25 and contain large proportions of acidic amino acids (19.7%-22.0%) in addition to hydrophobic amino acids (37.3%-38.5%). They share a novel amino-terminal sequence through at least 19 residues. Carbohydrate analysis using periodic acid-Schiff staining and treatment with trifluoromethanesulfonic acid shows no evidence of glycosylation. Antiserum to a purified Mr = 100,000 protein from C. fetus 82-40 LP cross-reacts with three other purified C. fetus ***surface*** -array proteins by enzyme-linked immunosorbent assay with titers greater than 12,800. We conclude that: 1) there is a family of ***surface*** -array proteins of C. fetus with common structural and antigenic characteristics; 2) that these molecules have similar biochemical characteristics to surface -array proteins described for other bacteria; but however, 3) by amino-terminal sequence analysis these are unique.
Descriptors: *Bacterial Proteins--isolation and purification--IP; * Campylobacter fetus--analysis--AN; *Membrane Glycoproteins--isolation and purification--IP; Amino Acid Sequence; Amino Acids--analysis--AN; Enzyme-Linked Immunosorbent Assay; Isoelectric Focusing; Molecular Sequence Data; Molecular Weight

Publishing Model Print
Document type: Journal Article; Research Support, U.S. Gov't, Non-P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

Infection with Campylobacter pyloridis has been strongly associated with gastritis in humans although its etiologic significance is currently undefined. We examined the structure and antigenicity of whole-cell, outer-membrane, acid-extractable surface protein, and proteinase K-treated whole cell lysate preparations from eight C. pyloridis strains by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting with homologous and heterologous immune rabbit serum. Whole-cell and outer-membrane profiles observed in all strains of C. pyloridis were nearly identical; none were similar to those of C. jejuni and C. fetus. Major whole-cell bands migrated at 26,000, 29,000, 56,000, and 62,000 molecular weights. The acid-extracted protein profiles of all C. pyloridis strains also were similar to one another and showed similarities with acid-extracted proteins from C. jejuni, with major bands migrating at 29,000, 48,000 to 53,000, and 62,000. All proteinase K-treated lysates showed different lipopolysaccharide (LPS) profiles, ranging from rough to smooth with multiple repeating side chains. Immunoblots of whole-cell and proteinase K-treated preparations of the C. pyloridis strains showed that there was antigenic cross-reactivity of proteins migrating at 62,000 and 56,000, but not in other regions, and cross-reactivity between LPS core regions but not side chains. These results suggest that C. pyloridis has both protein and core LPS group antigens and strain-specific protein and LPS side chain antigens.

Descriptors: *Antigens, Bacterial--immunology--IM; *Campylobacter --immunology--IM; Antibodies, Bacterial--immunology--IM; Antigens, Bacterial--isolation and purification--IP; Bacterial Proteins--immunology --IM; Bacterial Proteins--isolation and purification--IP; Campylobacter--classification--CL; Electrophoresis, Polyacrylamide Gel; Endopeptidase K; Endopeptidases; Lipopolysaccharides--immunology--IM; Lipopolysaccharides--isolation and purification--IP; Molecular Weight; Serotyping; Species Specificity

CAS Registry Number: 0 (Antibodies, Bacterial); 0 (Antigens, Bacterial); 0 (Bacterial Proteins); 0 (Lipopolysaccharides)

Enzyme Number: EC 3.4.- (Endopeptidases); EC 3.4.21.64 (Endopeptidase K)

Record Date Created: 19870605

Record Date Completed: 19870605

6/9/37 (Item 37 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07319206 PMID: 3819475

Pathogenesis of Campylobacter fetus infections: serum resistance associated with high-molecular-weight ***surface*** proteins.

Blaser M J; Smith P F; Hopkins J A; Heinzer I; Bryner J H; Wang W L

Journal of infectious diseases (UNITED STATES) Apr 1987, 155 (4) p696-706, ISSN 0022-1899--Print Journal Code: 0413675

Publishing Model Print
Document type: Journal Article; Research Support, U.S. Gov't, Non-P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: AIM; INDEX MEDICUS

Campylobacter fetus subspecies fetus causes both systemic and diarrheal illnesses. We studied 38 strains of C. fetus isolated from 34 patients; underlying illness was present in eight (89%) of nine patients with only systemic isolates compared with three (20%) of 15 patients with only fecal isolates (P = .002). In a standardized assay of susceptibility

to normal human serum, 27 (71%) strains were resistant, six (16%) had intermediate susceptibility, and five (13%) were serum sensitive. Major protein bands migrating at 100 kDa or 125 kDa on polyacrylamide gels were present in all of the 25 serum-resistant strains tested but in only four of seven serum-sensitive isolates of C. fetus from humans and animals (P = .007). The presence of these bands was associated with type A lipopolysaccharide. A low-passaged strain, 82-40, was serum resistant and contained the 100-kDa protein; however, a spontaneous mutant of this strain lacked this band and was serum sensitive. The 100-kDa and 125-kDa proteins of three strains of C. fetus were antigenically cross reactive or identical and were exposed on the ***surface*** of the C. fetus cell. Serum resistance is inherent to most C. fetus isolates from humans and is associated with the presence of cross-reactive ***surface*** proteins.

Tags: Female; Male

Descriptors: *Bacterial Outer Membrane Proteins--physiology--PH; *Blood Bactericidal Activity; *Campylobacter fetus--pathogenicity--PY; *Complement System Proteins--immunology--IM; Animals; Antigens, Bacterial --immunology--IM; Bacterial Outer Membrane Proteins--immunology--IM; Campylobacter Infections--microbiology--MI; Campylobacter fetus --genetics--GE; Campylobacter fetus--immunology--IM; Feces --microbiology--MI; Humans; Lipopolysaccharides--physiology--PH; Molecular Weight; Mutation

CAS Registry Number: 0 (Antigens, Bacterial); 0 (Bacterial Outer Membrane Proteins); 0 (Lipopolysaccharides); 9007-36-7 (Complement System Proteins)

Record Date Created: 19870417

Record Date Completed: 19870417

6/9/38 (Item 38 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07297045 PMID: 3274046

Heterozygosity at the Km locus associated with humoral immunity to ***Campylobacter*** jejuni.

Pandey J P; Blaser M J

Department of Basic and Clinical Immunology and Microbiology, Medical University of South Carolina.

Experimental and clinical immunogenetics (SWITZERLAND) 1986, 3 (1) p49-53, ISSN 0254-9670--Print Journal Code: 8411714

Contract/Grant Number: AI-18940; AI; NIAID; AM-24021; AM; NIADDK

Publishing Model Print

Document type: Comparative Study; Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Serum samples from 43 Caucasian subjects convalescing from acute Campylobacter jejuni infection were typed for nine Gm and two Km determinants. The sera were also used to measure IgA, IgG, and IgM classes of antibody to acid-labile ***surface*** proteins of C. jejuni by an enzyme-linked immunosorbent assay. A highly significant association (p = 0.004) was found between Km1/Km3 heterozygotes and the level of IgA antibodies. These results suggest the existence of complementary immune response genes which in the heterozygous condition permit a humoral response to C. jejuni.

Descriptors: *Antibodies, Bacterial--biosynthesis--BI; *Campylobacter%%% Infections--immunology--IM; *Immunoglobulin Km Allotypes--genetics--GE; Campylobacter Infections--genetics--GE; Campylobacter fetus --immunology--IM; Disease Susceptibility; Heterozygote; Humans; Immunoglobulin A--biosynthesis--BI; Immunoglobulin A--genetics--GE;

Immunoglobulin Gm Allotypes--genetics--GE
*** CAS Registry Number: 0 (Antibodies, Bacterial); 0 (Immunoglobulin A); 0***
(Immunoglobulin Gm Allotypes); 0 (Immunoglobulin Km Allotypes)
Record Date Created: 19900712
Record Date Completed: 19900712

6/9/39 (Item 39 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07074362 PMID: 3522430
Antigenicity of ***Campylobacter*** jejuni flagella.
Blaser M J; Hopkins J A; Perez-Perez G I; Cody H J; Newell D G
Infection and immunity (UNITED STATES) Jul 1986, 53 (1) p47-52, ISSN 0019-9567--Print Journal Code: 0246127
Publishing Model Print
Document type: Journal Article; Research Support, U.S. Gov't, Non-P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
We studied the antigenicity of a wild-type flagellate and motile (F+M+) Campylobacter jejuni strain (81116) and two daughter mutants, one flagellate and immotile (F+M-) and one aflagellate and immotile (F-M-). By sodium dodecyl sulfate-polyacrylamide gel electrophoresis of acid-extracted surface proteins, a 63-kilodalton (kDa) band identified from sheared flagella as the flagellar protein was present in the F+M+ and F+M- strains but not in the F-M- strain. No other differences in protein profile among the three strains were noted. By Western blotting, serum from rabbits immunized with either the F+M+ or F-M- strain detected a 63-kDa protein in the F+M+ and F+M- strains but not in the F-M- strain. That the F-M- antiserum recognized the 63-kDa band suggests that small amounts of this protein or a cross-reacting antigen is present on the F-M- strain. By counterimmunoelectrophoresis of the acid-extracted preparations with immune sera, all three strains were found to share three major antigens, but a fourth antigen with a net positive charge was present only in the F+M+ and F+M- strains. Antisera to five C. jejuni and two ***Campylobacter*** fetus strains recognized the 63-kDa protein of purified F+M+ flagella in Western blots, demonstrating a common antigen is present, but enzyme-linked immunosorbent assay results suggest that the sharing of this antigen among ***Campylobacter*** strains is variable.
Descriptors: *Antigens, Bacterial--immunology--IM; *Campylobacter fetus--immunology--IM; *Flagella--immunology--IM; Campylobacter fetus--pathogenicity--PY; Cell Fractionation--methods--MT; Counterimmunoelectrophoresis; Electrophoresis, Polyacrylamide Gel; Immunosorbent Techniques; Molecular Weight
CAS Registry Number: 0 (Antigens, Bacterial)
Record Date Created: 19860811
Record Date Completed: 19860811

6/9/40 (Item 40 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06998540 PMID: 3954344
Inactivation of Campylobacter jejuni by chlorine and monochloramine.
Blaser M J; Smith P F; Wang W L; Hoff J C
Applied and environmental microbiology (UNITED STATES) Feb 1986, 51 (2) p307-11, ISSN 0099-2240--Print Journal Code: 7605801
Publishing Model Print

Document type: Journal Article; Research Support, U.S. Gov't, Non-P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib

Campylobacter jejuni and closely related organisms are important bacterial causes of acute diarrheal illness in the United States. Both endemic and epidemic infections have been associated with consuming untreated or improperly treated ***surface*** water. We compared susceptibility of three C. jejuni strains and Escherichia coli ATCC 11229 with standard procedures used to disinfect water. Inactivation of bacterial preparations with 0.1 mg of chlorine and 1.0 mg of monochloramine per liter was determined at pH 6 and 8 and at 4 and 25 degrees C. Under virtually every condition tested, each of the three C. jejuni strains was more susceptible than the E. coli control strain, with greater than 99% inactivation after 15 min of contact with 1.0 mg of monochloramine per liter or 5 min of contact with 0.1 mg of free chlorine per liter. Results of experiments in which an antibiotic-containing medium was used suggest that a high proportion of the remaining cells were injured. An animal-passaged C. jejuni strain was as susceptible to chlorine disinfection as were laboratory-passaged strains. These results suggest that disinfection procedures commonly used for treatment of drinking water to remove coliform bacteria are adequate to eliminate C. jejuni and further correlate with the absence of outbreaks associated with properly treated water.

Descriptors: *Campylobacter--drug effects--DE; *Chloramines --pharmacology--PD; *Chlorine--pharmacology--PD; Animals; Campylobacter%%%--growth and development--GD; Campylobacter --isolation and purification--IP; Disinfection; Hydrogen-Ion Concentration; Kinetics; Mice; Temperature; Time Factors

*** CAS Registry Number: 0 (Chloramines); 10599-90-3 (chloramine); 7782-50-5***
(Chlorine)

Record Date Created: 19860423
Record Date Completed: 19860423

6/9/41 (Item 41 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06976343 PMID: 3944480

Immune response to Campylobacter jejuni in a rural community in Thailand.

Blaser M J; Taylor D N; Echeverria P

Journal of infectious diseases (UNITED STATES) Feb 1986, 153 (2) p249-54, ISSN 0022-1899--Print Journal Code: 0413675
Publishing Model Print
Document type: Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: AIM; INDEX MEDICUS

We studied the prevalence of antibodies to Campylobacter jejuni in serum from healthy Thai villagers by using enzyme-linked immunosorbent assays with C. jejuni ***surface*** proteins as antigens. Levels of C. jejuni-specific IgA rose progressively through life, IgG peaked in the second year of life and then fell, and IgM peaked during late childhood and the teenage years. These findings confirm results observed in Bangladeshi children, and they suggest there is intense early exposure and continued exposure through life. The ratio of C. jejuni-specific IgA to total IgA was constant in all age groups while the ratio of specific IgG and IgM followed the same age-related pattern as the levels of antibody to C. jejuni. The

age-related discordance between the C. jejuni-specific IgA and IgG levels observed in this and the previous study are at present unexplained.
Tags: Female; Male
Descriptors: *Antibodies, Bacterial--analysis--AN; *Campylobacter fetus--immunology--IM; Adolescent; Adult; Age Factors; Child; Child, Preschool; Enzyme-Linked Immunosorbent Assay; Fetal Blood--immunology--IM; Humans; Immunoglobulin A--analysis--AN; Immunoglobulin G--analysis--AN; Immunoglobulin M--analysis--AN; Infant; Thailand
CAS Registry Number: 0 (Antibodies, Bacterial); 0 (Immunoglobulin A); 0 (Immunoglobulin G); 0 (Immunoglobulin M)
Record Date Created: 19860228
Record Date Completed: 19860228

6/9/42 (Item 42 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06702580 PMID: 2580793
Antigenic heterogeneity of lipopolysaccharides from Campylobacter jejuni and ***Campylobacter*** fetus.
Perez G I; Hopkins J A; Blaser M J
Infection and immunity (UNITED STATES) May 1985, 48 (2) p528-33, ISSN 0019-9567--Print Journal Code: 0246127
Publishing Model Print
Document type: Comparative Study; Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
The lipopolysaccharide (LPS) structure of ***Campylobacter*** spp. can be visualized with polyacrylamide gel electrophoresis by examining proteinase K-treated whole cell lysates. Polyacrylamide gel electrophoresis LPS profiles of C. jejuni strains are rough type with low concentrations of low-molecular-weight polysaccharide side chains, serum-resistant C. fetus strains have smooth-type LPS, and serum-sensitive C. fetus strains have rough-type LPS. We electroblotted the proteinase K-treated whole cell lysates of 17 C. jejuni and 9 C. fetus strains from polyacrylamide gel electrophoresis to nitrocellulose paper to examine antigenicity to immune rabbit sera. There was virtually no antigenic cross-reactivity of C. jejuni and C. fetus LPS. Among C. jejuni strains, core LPS structures were cross-reactive, but the O-polysaccharide side chains were best recognized by homologous antisera. Antisera to several serum-resistant C. fetus strains recognized only the polysaccharide side-chain regions of serum-resistant strains and no part of the LPS from the sensitive strain. Antiserum raised against a serum-sensitive C. fetus strain but not homologous antisera recognized the core region of the LPS of the serum-resistant C. fetus strains. These findings suggest that core LPS antigens are widely shared within C. fetus subsp. fetus strains but that in the serum-resistant strains this core region is not surface exposed and therefore not immunogenic to rabbits infected with whole cells.
Descriptors: *Campylobacter fetus--immunology--IM; *Lipopolysaccharides--immunology--IM; Blood Bactericidal Activity; Cross Reactions; Electrophoresis, Polyacrylamide Gel; Epitopes; Immune Sera; Immunologic Techniques; Lipopolysaccharides--analysis--AN; Species Specificity
CAS Registry Number: 0 (Epitopes); 0 (Immune Sera); 0 (Lipopolysaccharides)
Record Date Created: 19850610
Record Date Completed: 19850610

6/9/43 (Item 43 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06201571 PMID: 6618667
Identification and characterization of Campylobacter jejuni outer membrane proteins.
Blaser M J; Hopkins J A; Berka R M; Vasil M L; Wang W L
Infection and immunity (UNITED STATES) Oct 1983, 42 (1) p276-84, ISSN 0019-9567--Print Journal Code: 0246127
Publishing Model Print
Document type: Journal Article; Research Support, U.S. Gov't, Non-P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

Outer membrane proteins from isolates of Campylobacter jejuni were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Sarcosinate-insoluble membrane preparations were outer membrane enriched based on increased ketodeoxyoctonate concentrations, the presence of surface -exposed 125I-labeled proteins that were hydrophobic, and similarity to membrane vesicle (bleb) sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles. Most isolates contained a single major band with molecular weight of 41,000 to 45,000. Profiles of C. jejuni and Campylobacter coli isolates were indistinguishable, but either could be easily differentiated from Campylobacter fetus and ***Campylobacter*** faecalis. The profiles were stable for strains under a variety of growth, incubation and passage conditions. We classified 110 isolates from patients with sporadic campylobacter enteritis into nine subtypes based on differences in outer membrane sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles. Two categories accounted for 76% of the isolates. Complete concordance was observed in subtypes of strains obtained from epidemiologically related cases. Thus, comparison of the major outer membrane proteins of C. jejuni is a useful technique for investigating the transmission of this organism and may provide a basis for immunological characterization of the outer membrane proteins.

Descriptors: *Bacterial Proteins--analysis--AN; *Campylobacter fetus--analysis--AN; *Membrane Proteins--analysis--AN; Bacterial Outer Membrane Proteins; Campylobacter--analysis--AN; Campylobacter fetus--classification--CL; Electrophoresis, Polyacrylamide Gel; Molecular Weight; Species Specificity; Sugar Acids--analysis--AN
CAS Registry Number: 0 (Bacterial Outer Membrane Proteins); 0 (Bacterial Proteins); 0 (Membrane Proteins); 0 (Sugar Acids); 1069-03-0 (2-keto-3-deoxyoctonate)
Record Date Created: 19831123
Record Date Completed: 19831123

6/9/44 (Item 44 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06123321 PMID: 6859722
Campylobacter enteritis from untreated water in the Rocky Mountains.
Taylor D N; McDermott K T; Little J R; Wells J G; Blaser M J
Annals of internal medicine (UNITED STATES) Jul 1983, 99 (1) p38-40, ISSN 0003-4819--Print Journal Code: 0372351
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: AIM; INDEX MEDICUS

During the summers of 1980 and 1981 Campylobacter jejuni was

isolated from 23% and Giardia lamblia was isolated from 8% of persons with diarrheal disease acquired in the area of Grand Teton National Park, Wyoming. ***Campylobacter*** enteritis occurred most frequently in young adults who had been hiking in wilderness areas and was significantly associated with drinking untreated surface water in the week before illness (p less than 0.02 in 1980; p less than 0.005 in 1981). Penner serotype 4 was the commonest serotype isolated from humans and the only serotype isolated from an implicated mountain stream. These studies show that backcountry ***surface*** water can be an important source of C. jejuni and that infection with ***Campylobacter*** , as well as G. lamblia, should be considered as a cause of diarrhea in those who have recently returned from wilderness areas.
Tags: Female; Male
Descriptors: *Campylobacter Infections--etiology--ET; *Diarrhea --microbiology--MI; *Water Microbiology; Adult; Animals; Campylobacter Infections--epidemiology--EP; Campylobacter Infections--veterinary--VE; Campylobacter fetus --isolation and purification--IP; Feces--microbiology--MI; Horses--microbiology--MI; Humans ; Serotyping; Wyoming
Record Date Created: 19830729
Record Date Completed: 19830729
? t s6/3,kwic/58 76 85 90 100

6/3,KWIC/58 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11107093 BIOSIS NO.: 199243075684
IDENTIFICATION AND CHARACTERIZATION OF MAJOR COMMON ANTIGENS FROM CAMPYLOBACTER-JEJUNI
BOOK TITLE: NACHAMKIN, I., M. J. BLASER AND L. S. TOMPKINS (ED.). CAMPYLOBACTER JEJUNI: CURRENT STATUS AND FUTURE TRENDS; SYMPOSIUM HELD BY THE NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES. XI+300P. AMERICAN SOCIETY FOR MICROBIOLOGY: WASHINGTON, D.C., USA. ILLUS
AUTHOR: PEI Z (Reprint); ELLISON R T III; BLASER M J
AUTHOR ADDRESS: DIV INFECT DIS, DEP MED, VANDERBILT UNIV SCH MED, NASHVILLE, TENN 37232-2605, USA**USA
p236-237 1992
ISBN: 1-55581-042-X
DOCUMENT TYPE: Book; Meeting
RECORD TYPE: Citation
LANGUAGE: ENGLISH

IDENTIFICATION AND CHARACTERIZATION OF MAJOR COMMON ANTIGENS FROM CAMPYLOBACTER-JEJUNI
BOOK TITLE: NACHAMKIN, I., M. J. BLASER AND L. S. TOMPKINS (ED.). CAMPYLOBACTER JEJUNI: CURRENT STATUS AND FUTURE TRENDS; SYMPOSIUM HELD BY THE NATIONAL INSTITUTE OF ALLERGY AND...
...AUTHOR: ***BLASER M J***
DESCRIPTORS: CAMPYLOBACTER-COLI CAMPYLOBACTER-FETUS CAMPYLOBACTER-LARI HELICOBACTER-PYLORI PEB1 PEB2 PEB3 PEB4 VACCINE CANDIDATE DIAGNOSTIC POTENTIAL

6/3,KWIC/76 (Item 4 from file: 654)
DIALOG(R)File 654:US PAT.FULL.

4112660
Derwent Accession: 1995-106682
Utility
EXPIRED
C/ Campylobacter jejuni antigens and methods for their production and use

; DNA SEQUENCE WITH LEADER SEQUENCE CODING FOR PEB1A ANTIGEN FOR GENE EXPRESSION SYSTEMS; BACTERICIDES AND TREATMENT FOR DIARRHEAL DISEASE

Inventor: ***Blaser, Martin J.*** , Nashville, TN
Pei, Zhiheng, Nashville, TN
Assignee: Enteric Research Laboratories(02)
Enteric Research Laboratories Inc (Code: 37197)
Examiner: Caputa, Anthony C. (Art Unit: 187)
Law Firm: Ostrolenk, Faber, Gerb & Soffen, LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5874300	A	19990223	US 95402804	19950313
Continuation	Abandoned			US 93114420	19930830
CIP	Abandoned			US 93112387	19930827

Fulltext Word Count: 13461

Campylobacter jejuni antigens and methods for their production and use...
Inventor: ***Blaser, Martin J*** ...

Abstract:
...present invention provides an isolated nucleic acid encoding an approximately 26 kilodalton antigen, PEB1A, of Campylobacter jejuni, or an antigenic fragment thereof, wherein the antigen is associated with diarrheal disease. The present invention also provides methods of detecting the presence of a Campylobacter jejuni strain possessing the PEB1A antigen in a subject. Vaccines and treatments for C. jejuni...

Summary of the Invention:
...This invention relates to the ***Campylobacter*** jejuni antigen PEB1A, to nucleic acid encoding the antigen, to various methods of detecting Campylobacter jejuni infection, and to vaccines and treatments for ***Campylobacter*** jejuni enteritis. In particular, the interaction of the PEB1A antigen with its receptor can be...

... ***Campylobacter*** jejuni is now recognized as one of the leading causes of diarrheal diseases worldwide. Approximately...

...In one embodiment, the invention provides an isolated nucleic acid encoding a PEB1A antigen, Campylobacter jejuni or antigenic fragment thereof. In preferred embodiments, the nucleic acid comprises nucleotides 25 through...

...In another aspect of the invention, a method is provided for detecting the presence of Campylobacter jejuni infection comprising contacting an antibody-containing sample obtained from a patient suspect of infection...

...a sufficient time to allow formation of a complex between the polypeptide and any anti-Campylobacter jejuni antibodies present in the sample, formation of complex is measured by standard techniques. In ...

...In an alternative method of detecting the presence of Campylobacter jejuni, a sample is obtained from a patient suspected of infection and is contacted with...

...ID. NOS: 1, 3, 5, 7 and 9, respectively). It contains a coding region for ***peb*** 1A, generally open reading frame "D" (appearing SEQ. ID. NO: 7). Each of SEQ. ID

Description of the Drawings:
...FIG. 4A is a PCR amplification of 702 bp peb1A fragment from ***Campylobacter*** strains. Lanes are: C. jejuni strains 81-176 (a), D1916 (b), 85AC (c); C. coli...

...PCR product was found in all C. jejuni strains (arrow) but not in the other ***Campylobacter*** species...

Description of the Invention:
...the expression of human gamma-interferon, tissue plasminogen activator, clotting Factor VIII, hepatitis B virus surface antigen, protease Nexinl, and eosinophil major basic protein. Further, the vector can include CMV promoter...176 (ATCC 55026) was used to clone the gene for the PEB1A antigen. Twelve clinical ***Campylobacter*** isolates from humans, including 5 C. jejuni, 3 C. coli, 2 C. lari, and 2...

...degree(s)] C. in Brucella broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 15% glycerol. ***Campylobacter*** strains were cultured in Brucella broth supplemented with 5% sheep blood in a microaerobic atmosphere...

... ***Campylobacter*** chromosomal DNA was digested with HindIII and the resulting fragments were electrophoresed on a 0...

... ***Campylobacter*** strains were grown on trypticase soy blood agar plates (BBL) and replica copies of these...

...We next sought to determine the conservation of peb1A among Campylobacter strains by Southern hybridization since PEB1A is apparently present in all C. jejuni strains examined...

...HindIII-digested chromosomal fragment from all three C. jejuni strains but not to the other ***Campylobacter*** strains examined (FIG. 3). When the same pair of primers was used in PCR analysis...

...Construction and characterization of a PEB1A-negative strain of Campylobacter jejuni Bacterial strains, vectors and growth conditions...

...ATCC 55026) used in this study was from the culture collection of the Vanderbilt University Campylobacter/Helicobacter Laboratory and was chosen because it has been extensively characterized. Stock cultures were maintained

6/3,KWIC/85 (Item 13 from file: 654)
DIALOG(R)File 654:US PAT.FULL.

3370999
Derwent Accession: 1992-199949
Utility
REASSIGNED, EXPIRED
C/ Diagnostic testing for Campylobacter jejuni or Campylobacter coli infections using novel antigens
; CONTACTING WITH ANTIBODIES; COMPLEXING; COMPARING TO PREDETERMINED THRESHOLD LEVEL
Inventor: ***Blaser, Martin J.*** , 733 Darden Place, Nashville, TN, 37205
Ellison, III, Richard T., 2391 Eudora St., Denver, CO, 80207
Pei, Zhi H., 2139-K Acklen Ave., Nashville, TN, 37212
Assignee: Unassigned
Unassigned Or Assigned To Individual (Code: 68000)
Examiner: Kepplinger, Esther L. (Art Unit: 182)

Assistant Examiner: Bidwell, Carol E.
Law Firm: Ostrolenk, Faber, Gerb & Soffen

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5200344	A	19930406	US 90612330	19901113

Fulltext Word Count: 9100

Diagnostic testing for Campylobacter jejuni or Campylobacter coli infections using novel antigens...
Inventor: ***Blaser, Martin J*** ...

Abstract:
Antigenic compositions useful in diagnostic testing for the presence of Campylobacter jejuni or Campylobacter coli comprising a PEB1 antigen obtained from Campylobacter jejuni having an apparent molecular weight of about 28 kDa as measured by SDS-PAGE...

...gel filtration chromatography under native conditions and an isoelectric point of 8.5 or a ***PEB3*** antigen obtained from ***Campylobacter*** jejuni having an apparent molecular weight of about 30 kDa as measured by SDS-PAGE...

Summary of the Invention:
...This invention relates to novel antigenic compositions useful in diagnostic testing for the presence of ***Campylobacter*** jejuni ("C. jejuni") or ***Campylobacter*** coli ("C. coli") infection, and useful as vaccines for providing immunological protection against such infection...

...C. coli are believed to cause both inflammatory and non-inflammatory gastroenteritis (Blaser et al., " ***Campylobacter*** Enteritis," N. Eng. J. Med., 1981; 305:1444-1452). They are recognized as leading causes of inflammatory diarrhea in the U.S. and other developed countries (Blaser et al., ***Campylobacter*** Enteritis in the United States: "A Multicenter Study," Ann. Intern. Med., 1983;98:360-365...

...diarrheal disease in the developing world (Glass et al., "Epidemiological and Clinical Features of Endemic Campylobacter Jejuni infection in Bangladesh," J. Infect. Dis., 1983;148:292-296). They have further been...

...organisms are readily killed by pasteurization, many persons consume unpasteurized milk, especially in rural areas. ***Surface*** water may be contaminated with C. jejuni or C. coli, and persons who consume such...
...raised poultry are particularly susceptible to contamination with these pathogens (Blaser et al., Epidemiology of ***Campylobacter*** Jejuni Infections, Epidemiologic Reviews, 1983;5:157-176). Many of the animals used as pets...

...coli develop antibodies specific to the organisms (Blaser et al., "Human Serum Antibody Response to Campylobacter Jejuni as Measured in an Enzyme-Linked Immunosorbent Assay", Infect. Immun.. 1984;44:292-298...

...C. jejuni infection offers some protection against subsequent C. jejuni disease (Black et al., "Experimental ***Campylobacter*** Jejuni Infection in Humans," J. Infect. Dis., 1988;157:472-479; Perlman et al., "Humoral Immune Response to Campylobacter Jejuni in Human Volunteers," Abstract presented at the 87th Annual Meeting of the American Society for Microbiology, Atlanta, GA, March 1987; Perlman et al., "Immunity to Campylobacter Jejuni Following Oral Challenge to Volunteers," Abstract presented at the Fourth International Workshop on

Campylobacter Infections, Goteborg, Sweden, June 1987.) Similarly, individuals with prior exposure to unpasteurized milk and high...

...contaminated with C. jejuni (Blaser et al., "The Influence of Immunity on Raw-Milk Associated Campylobacter Infections," JAMA 1987;257:43-46.) However, while this work indicates that exposure to whole...

...Certain ***surface*** proteins of C. fetus are disclosed by "Purification and Characterization of a Family of High Molecular Weight Cell ***Surface*** Proteins from ***Campylobacter*** Fetus," J. Biol. Chem., 1988;263:6416-6420...

...18, 1988, Blaser et al. disclose antigenic compositions for use in detecting antibodies specific for Campylobacter [Helicobacter] pylori...

...weights of 29 kDa, 30 kDa and 31 kDa are discussed in Blaser et al., " Campylobacter jejuni outer membrane proteins are antigenic for humans," Infection and Immunity, Vol. 43, No. 3...

...be immunogenic by immunoblotting in Dunn et al., "Two-Dimensional Gel Electrophoresis and Immunoblotting of Campylobacter Outer Membrane Proteins," Infection and Immunity, Vol. 55, No. 7 pp. 1564-72 (July 1987 ...

...U.S. Pat. No. 4,882,271 discloses a 300-700 kDa antigen from ***Campylobacter*** pylori and its use in various assays...

...In one embodiment of the invention, an antigenic composition comprises at least one of two Campylobacter jejuni and Campylobacter coli-specific antigens, both of said antigens being obtainable by acid extraction of ***surface*** antigens C. jejuni, one of said antigens (hereinafter "PEB1" which term includes antigen fragments of...

...under native conditions) and an isoelectric point of about 8.5, the other of said surface antigens (hereinafter "PEB3" which term includes antigenic fragments of the natural protein whether derived from the natural protein...

...in said antigenic composition at a concentration higher than that resulting from acid extraction of surface antigens from whole cell ***Campylobacter*** jejuni. These antigens (PEB1 and ***PEB3***) are highly conserved, and have strong affinity for antibodies induced by most animals' immune response...

...In preferred embodiments, each acid extractable ***surface*** antigen (PEB1 and PEB3) is present in the antigenic composition at a concentration, relative to other acid extractable materials...
...Antigenic proteins having substantial homology to said PEB1 and/or PEB3 antigens or their fragments may also be used in accordance with the invention...

...PEB1 and/or ***PEB3*** antigens described above may be capable of inducing protective immunity against both C. jejuni and...

...In one aspect of the invention, antigenic compositions containing the PEB1 and/or PEB3 antigens described above are used in methods for the detection of C. jejuni- or C...

...Antisera raised against the PEB1 and/or ***PEB3*** antigen described above may be used in a particularly sensitive and specific test for presence...

Description of the Drawings:

...for the presence of PEB1 antigen by SDS-PAGE. Peak 2 contained a mixture of PEB3 and PEB4 antigens, and Peak 3 contained PEB1 antigen with greater than 98% purity...

...81-176. Lanes are whole bacterial cells(WC), acid extract(AE), PEB1(28K), PEB2(29K), ***PEB3*** (30K) and PEB4(31K) antigens. Molecular weight markers are shown at left...

...FIG. 3 is a graph showing recognition of ***Campylobacter*** and Helicobacter by antisera to C. jejuni proteins, by ELISA. Whole bacterial cells were used...

...FIG. 4 is a graph showing recognition of ***Campylobacter*** and Helicobacter by antisera to C. jejuni proteins, by ELISA. Whole bacterial cells were used...

...FIG. 5 is a Western blot of anti-PEB1 against representative ***Campylobacter*** and Helicobacter strains. The antigens used are whole cells prepared as described in EXAMPLES 2...

...DNA hybridization. The C. fetus strains were identified by the presence of high molecular weight surface array proteins detected by SGS-PAGE and Western blot (Z. Pei and M. Blaser J...

Description of the Invention:

...The PEB1 and ***PEB3*** antigens from the deposited strain are found in all C. jejuni strains we have tested...

...concentrations were measured using the Markwell et al. modification of the Lowry method for crude ***surface*** protein (Markwell et al. A modification of the Lowry procedure to simplify protein determinations in ...

...water by centrifugation at 3500X g for 15 min. To prepare a crude mixture of surface structures, the bacterial cells were suspended in 0.2 M glycine-hydrochloride buffer, pH 2...

...For purification of ***PEB3*** antigen, the acid extracted material was separated by cationic exchange FPLC chromatography on a Mono...

...mM over 20 minutes. Each fraction was checked by SDS-PAGE for the presence of ***PEB3*** migrating at 30 kDa. The ***PEB3*** antigen eluted from the column at about 200 mM NaCl. The fraction containing partially purified PEB3 was purified to homogeneity on the phenyl-Superose column using the same conditions as described above for the purification of the PEB1 antigen. ***PEB3*** eluted from this column at around 450 mM Na[sub]2 SO[sub]4. Purified ***PEB3*** (30 kDa) is shown in FIG. 2...

...the PEB1 antigen, the pI was found to be about 8.5, and for the ***PEB3*** antigen, the pI was greater than 9.3...

...involve the presence of fluorescein, an enzyme or a substrate inside a liposome onto whose ***surface*** C. jejuni antigens are expressed These liposomes are incubated with a diluted body fluid sample to be tested, and are thoroughly washed Any liposomes with immunoglobulins on their surface forming an antigen/antibody complex may be recognized by attaching a second antibody, specific to...

...Determination of the antigenicity of the PEB1 and ***PEB3*** proteins for infected humans using an ELISA...

...The purified PEB1 and ***PEB3*** proteins were compared with a crude acid-extracted mixture of C. jejuni proteins as antigens...

...followed the teachings of Blaser et al. (Blaser et al., "Human serum antibody response to Campylobacter jejuni as measured in an enzyme-linked immunosorbent assay," Infect Immun., 1984;44:292-298...

...To sensitize ELISA plates (Nunc, Inc., Naperville, IL), purified proteins and the crude surface protein preparation were diluted in 0.015 M carbonate buffer, pH 9.6. One hundred...

...five strains of C. jejuni, 15 of C. coli, 10 of C. fetus, 5 of Campylobacter laridis and 5 of Helicobacter pylori (formerly known as C. pylori) were used in this...

...defined as positive. In this system normal rabbit serum did not recognize any of the ***Campylobacter*** strains, as expected (Table 4). In contrast, the antisera to the mixture of acid-extracted...

...35 C. jejuni strains, and all 15 C. coli strains, but none of the other ***Campylobacter*** or Helicobacter isolates (Table 4 and FIG. 3). Thus, the antisera to the PEB1 protein...

...To further confirm the specificity of recognition of ***Campylobacter*** strains in ELISA by antiserum to PEB1 antigen in Example 2, we performed Western blotting...

...for the bands recognized by this serum in preparations of whole bacterial cells of various ***Campylobacter*** and Helicobacter species. Whole bacterial cells were prepared as described in Example 2. 0.5...

...resolve is whether PEB1 antigen is a protein. To answer this, 24-hour cultures of Campylobacter strains on blood agar plates were harvested in sterile distilled H[sub]2 O (5...

...We have considered the potential application of the use of the PEB1 and/or ***PEB3*** antigens in the development of a vaccine against C. jejuni and C. coli infections. To...

...of gastric acid and proteolytic enzymes on the vaccine preparation, the whole PEB1 and/or PEB3 antigen (or a fragment of one or both of these proteins) will be packaged either...

...0 mg of the antigens of the invention, which may be either pure PEB1, pure PEB3, or a mixture of PEB1 and PEB3, for example, a dosage of about 10.0 mg of pure or mixed antigen...

...To enhance delivery of PEB1 and/or ***PEB3*** to the gastrointestinal immune system the protein(s) [or a fragment(s) of the proteins...

...For C. jejuni, a parenteral vaccine could include PEB1 and/or ***PEB3*** or fragments thereof. The protein(s) or fragment(s) could be administered with an adjuvant...

...to humans as 1.0 mg (range 0.5-5.0 mg) of antigen (PEB1, ***PEB3*** , or mixture of both) in 1 ml of phosphate buffered saline (pH7.4). With a...

...containing a plurality of wells, plates which were coated prior to use with PEB1 or PEB3 antigens, and ELISA materials for enzyme detection consisting of peroxidase-labeled goat anti-human IgG...

...contains peroxidase-labelled goat or rabbit anti-human immunoglobulin and a source of PEB1 or ***PEB3*** antigens...

...detecting antibodies using the indirect immunofluorescence assay may include a compartmental container with PEB1 or PEB3 antigens, human test serum, phosphate buffered saline and fluorescein-conjugated goat anti-human IgG...

...serum, fluorescent marker- (or enzyme- or substrate-) filled liposome with C. pylori antigens on their ***surface*** , and a ***surface*** -active agent. In this assay the container might be a precoated tube or well with...
...the latex agglutination assay may include a compartmental container, hyperimmune serum to PEB1 and/or PEB3 conjugated to latex beads, and phosphate buffered saline or water...

...or C. coli organisms in fecal or water specimens, fecal or water specimens enriched for Campylobacter by selective enrichment methods, or in colonies on solid media suspected as being C. jejuni...

Exemplary or Independent Claim(s):
1. A method for detecting the presence of antibodies to Campylobacter jejuni or Campylobacter coli comprising contacting a test sample suspected of containing said antibodies with an amount of...

...said antigenic composition comprising a PEB1 antigen which, when obtained from Campylobacter jejuni without alteration of its natural composition, has an apparent molecular weight of about 28...

...antigen being present in said antigenic composition at a concentration, relative to other acid-extractable surface structures of Campylobacter jejuni, higher than that resulting from acid extraction of surface structures from whole cell ***Campylobacter*** jejuni...

...8. A method for detecting the presence of antibodies to Campylobacter jejuni or Campylobacter coli comprising contacting a test sample suspected of containing said antibodies with an amount of...
...said antigenic composition comprising a ***PEB3*** antigen which, when obtained from Campylobacter jejuni without alteration of its natural composition, has an apparent molecular weight of 30 kDa...

...sulfate polyacrylamide gel under reducing conditions), and an isoelectric point greater than 9.3, said ***PEB3*** antigen being present in said antigenic composition at a concentration, relative to other acid-extractable surface structures of Campylobacter jejuni, higher than that resulting from acid extraction of surface structures from whole cell ***Campylobacter*** jejuni.

Non-exemplary or Dependent Claim(s):
2. The method of claim 1, wherein said antigenic composition further comprises a PEB3 antigen which, when obtained from Campylobacter jejuni without alteration of its natural composition, has an apparent molecular weight of about 30...

...isoelectric point greater than 9.3, said PEB1 antigen being present in said antigenic extractable surface structures of Campylobacter jejuni, higher than that resulting from acid-extraction of surface structures from whole cell ***Campylobacter*** jejuni or ***Campylobacter*** coli...

...7. A method of determining the presence of ***Campylobacter*** jejuni or Campylobacter coli in a test sample comprising the steps of contacting said test sample with an antibody-containing composition for a time sufficient to allow said antibodies to bind Campylobacter jejuni or Campylobacter coli, if present in said sample to form an organism/antibody complex, and then determining...

...containing composition comprising immunoglobulin from antisera raised against a PEB1 antigen which, when obtained from Campylobacter jejuni without alteration of its natural composition, has an apparent molecular weight of about 28...

...13. A method of determining the presence of ***Campylobacter*** jejuni or Campylobacter coli in a test sample comprising the steps of contacting said test sample with an antibody-containing composition for a time sufficient to allow said antibodies to bind Campylobacter jejuni or Campylobacter coli, if present in said sample, to form an organism/antibody complex, and then determining...
...said antibody-containing composition comprising immunoglobulin from antisera raised against a PEB3 antigen which, when obtained from Campylobacter jejuni without alteration of its natural composition, has an apparent molecular weight of 30 kDa...

6/3,KWIC/90 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0228401 DBR Accession No.: 98-09998 PATENT
Mutant Campylobacter fetus encoding heterologous protein or encoding only one sapA homolog - useful in vaccines, e.g. against infectious abortion or infertility in ungulates, and are only briefly maintained in the host
AUTHOR: Blaser M J; Thompson S A; Dworkin J
CORPORATE SOURCE: Nashville, TN, USA.
PATENT ASSIGNEE: Univ.Vanderbilt 1998
PATENT NUMBER: WO 9833386 PATENT DATE: 980806 WPI ACCESSION NO.: 98-437061 (9837)
PRIORITY APPLIC. NO.: US 36321 APPLIC. DATE: 970131
NATIONAL APPLIC. NO.: WO 98US1780 APPLIC. DATE: 980130
LANGUAGE: English

Mutant Campylobacter fetus encoding heterologous protein or encoding only one sapA homolog
AUTHOR: Blaser M J; Thompson S A; Dworkin J
ABSTRACT: A mutant Campylobacter fetus strain (A) is claimed, which includes a DNA cassette encoding a heterologous protein (I...

...variation which normally occurs at a very low frequency, and only one of the S(surface)-layer proteins (SLP) encoded by one sapA analog is produced; (2) a mixture of C...

... to generate mucosal and systemic immune responses against (I), particularly an immunogen derived from Salmonella, Campylobacter jejuni, Escherichia coli 0157:H7, HIV virus or SIV virus or other pathogens, or against...
DESCRIPTORS: mutant Campylobacter fetus, DNA cassette, recA mutation, DNA rearrangement, reduced sapA expression, sapA chimera, sapCDEF expression mutant, appl. Salmonella, ***Campylobacter*** jejuni, Escherichia coli, HIV virus, SIV virus, C. fetus recombinant vaccine prepare, prevent ungulate infectious...

6/3,KWIC/100 (Item 8 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00211278
DIAGNOSTIC TESTING FOR CAMPYLOBACTER JEJUNI OR COLI INFECTION USING

ANTIGENS
TEST DE DIAGNOSTIC D'INFECTIONS PAR CAMPYLOBACTER JEJUNI OU COLI
UTILISANT DES ANTIGENES
Patent Applicant/Assignee:
BLASER Martin J,
Inventor(s):
BLASER Martin J,
PEI Zhiheng,
ELLISON Richard T III,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9208485 A1 19920529
Application: WO 91US8220 19911105 (PCT/WO US9108220)
Priority Application: US 90330 19901113
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(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AT AU BE BR CA CH DE DK ES FR GB GR IT JP LU NL SE
Publication Language: English
Fulltext Word Count: 11828
DIAGNOSTIC TESTING FOR CAMPYLOBACTER JEJUNI OR COLI INFECTION USING
ANTIGENS
TEST DE DIAGNOSTIC D'INFECTIONS PAR CAMPYLOBACTER JEJUNI OU COLI
UTILISANT DES ANTIGENES
Inventor(s):
BLASER Martin J...
Fulltext Availability:
Detailed Description
Claims

English Abstract
An antigenic composition includes antigens obtainable from Campylobacter jejuni and may be used as a vaccine to induce protective antibodies against both Campylobacter coli and ***Campylobacter*** jejuni. The antigenic composition, and antisera specific to the antigens can be used to detect Campylobacter coli or ***Campylobacter*** jejuni infection. Diagnostic detection kits include the novel antigenic composition or antisera thereto.

French Abstract
Une composition antigenique comprend des antigenes pouvant etre obtenus a partir de Campylobacter jejuni et peut etre utilisee comme vaccin pour induire des anticorps protecteurs contre a la fois ***Campylobacter*** coli et ***Campylobacter*** jejuni. La composition antigenique ainsi que des antiserums specifiques des antigenes peuvent etre utilises afin de detecter des infections par Campylobacter coli ou ***Campylobacter*** jejuni. Les materiels de detection permettant un diagnostic comprennent la nouvelle composition antigenique ou des...

Detailed Description
... C. coli are believed to cause both inflammatory and non-inflammatory gastroenteritis (Blaser et al., " ***Campylobacter*** Enteritis," N. Enge Jo Med., 1981; 305:1444@1452). They are recognized as leading causes of inflammatory diarrhea in the U.S. and other developed countries (Blaser et al., ***Campylobacter*** Enteritis in the United States: A Multicenter Study,' Ann. Intern. Med., 1983;98:360-365...

...diarrheal disease in the developing world (Glass et al., "Epidemiological and Clinical Features of Endemic Campylobacter Jejuni infection in Bangladesh," J. Infect. Dis., 1983;148:292@296). They have further been...

...organisms are
readily killed by pasteurization, many persons consume unpasteurized milk, especially in rural areas. ***Surface*** water may be contaminated with C. ieluni or C. coli, and persons who consume such...
...raised poultry are particularly
susceptible to contamination with these pathogens (Blaser et alo, Epidemiology of Campylobacter Jejuni Infec-'Zions, EDidemiologic Reviews, 1983;5:157@176).

Many of the animals used as...

...coli develop antibodies specific to the
organisms (Blaser et al., "Human Serum Antibody Response to Campylobacter Jejuni as Measured in an Enzyme@Linked Immunosorbent Assay", Infect. Immun.4 1984;44:292...C. jejuni infection offers some protection against subsequent =C. disease, (Black et al.,
. jejuni
"Experimental Campylobacter Jejuni Infection in Humans," J. Infect. Dis., 1988;157:472-479; Perlman et al., "Humoral Immune Response to ***Campylobacter*** Jejuni in Human Volunteers," Abstract presented at the 87th Annual Meeting of the American Society for Microbiology, Atlanta, GA. March 1987; Perlman et al., *Immunity to Campylobacter Jejuni Following Oral Challenge to Volunteers,* Abstract presented at the Fourth International Workshop on Campylobacter infections, Goteborg, Sweden, June 1987.) Similarly, individuals with prior exposure to unpasteurized milk and.high...

...contaminated with
C. jejuni (Blaser et al., *The Influence of Immunity on Raw@Milk Associated Campylobacter Infections,' JAMA 1987;257:43@46.) However, while this work indicates that exposure to whole...

...use of an antigen
capable of inducing the desired immune response in most recipients.

Certain ***surface*** proteins of C. fetus are disclosed by 'Purification and Characterization of a Family of High Molecular Weight Cell Surface Proteins from ***Campylobacter*** Fetus," J. Biol. Chemical, 1988;263:6416@6420.

In Miotti, 'Rapid Methods for the Molecular...
...18, 1988,
Blaser et al. disclose antigenic compositions for use in detecting antibodies specific for Campylobacter [Helicobacterl -oviori.

Outer membrane proteins of C. Jejuni having molecular weights of 29 kDa, 30 kDa and 31 kDa are discussed in Blaser et al., " ***Campylobacter*** jejuni outer membrane proteins ...be
immunogenic by immunoblotting in Dunn et al., "Two Dimensional Gel Electrophoresis and Immunoblotting of Campylobacter Outer membrane Proteins," Infection and Immunity, Vol, 55, Number 7 ppe 1564@72 (July 1987...

...Jejuni.

U.S. Patent Number 4,882,271 discloses a 300 700 kDa antigen from Campylobacter pylori and its use in various assays.

SUMMARY OF THE INVENTION

It is an object...

...in one embodiment of the invention, an antigenic composition comprises at least one of two Campylobacter leluni and Cam,pylobacter colispecific antigens, both of said antigens being obtainable by acid extraction of surface antigens C. jejuni, one of said antigens (hereinafter "PEBI" which term includes antigen fragments of...

...chromatography under native conditions) and an isoelectric point of about 8 the other of said surface antigens (hereinafter 'PEB3" which term includes antigenic fragments ...in said antigenic composition at a concentration higher than that resulting from acid extraction of surface antigens from whole cell Camvylobacter jejuni. These antigens (PEB1 and ***PEB3***) are highly conserved, and have strong affinity for antibodies induced by most animals' immune response...

...present in the body fluids of non@infected individuals.

In preferred embodiments, each acid extractable surface antigen (PEB1 and PEB3) is present in the antigenic composition at a concentration, relative to other acid extractable materials...

...four times the natural concentration.

Antigenic proteins having substantial homology to said PEBI and/or PEB3 antigens or their fragments may also be used in accordance with the invention.

PEBI and/or PEB3 antigens described above may be capable of inducing protective immunity against both C. leluni and...361@363).

In one aspect of the invention, antigenic compositions containing the PEBI and/or PEB3 antigens described above are used in methods for the detection of C. leluni@ or C...

...may become bound to antigens in said composition.

Antisera raised against the PEB1 and/or PEB3 antigen described above may be used in a ...for the presence of PEB1 antigen by SDS@ PAGE. Peak 2 contained a mixture of ***PEB3*** and PEB4 antigens, and Peak 3 contained PEB1 antigen with greater than 98% purity, Figure...

...176. Lanes are whole bacterial cells(WC), acid extract(AE), PEB1(28K), PEB2(29K)t ***PEB3*** (30K) and PEB4(31K) antigens. Molecular

weight markers are shown at left,
Figure 3 is...

...1 was defined as
414
positive.

Figure 4 is a graph showing recogni-tion of
Campylobacter and Helicobacter by antisera to C. leluni
proteins, by ELISA. Whole bacterial cells were used...

...DNA
hybridization. The C. fetus strains were identified by
the presence of high molecular weight surface array
proteins detected by SGS@PAGE and Western blot (Z. Pei
and M. Blaser J...patent arising from the present patent application
which refers to the deposit,
The PEB1 and PEB3 antigens from the
deposited strain are found in all C. jejuni strains we
have tested al. modification of the
Lowry method for crude ***surface*** protein (Markwell et al.

A modification of the Lowry procedure to simplify
protein determinations in...

...by centrifugation at 3500 X g for 15
min, To prepare a crude mixture of surface structures,
the bacterial cells were suspended in 0.2 M glycine
hydrochloride buffer, pH 2...

...minutes at a sodium sulfate concentration of about 390
mm (Figure 1).

For purification of PEB3 antigen, the acid
extracted material was separated by cationic exchange
FPLC chromatography on a Mono...

...MM over 20 minutes,
Each fraction was checked by SDS-PAGE for the presence
of ***PEB3*** migrating at 30 kDa. The ***PEB3*** Antigen eluted
from the column at about 200 mM NaC1. The fraction
containing partially purified PEB3 was purified to
homogeneity on the phenyl@Superose column using the
same conditions as described above for the purification
of the PEB1 antigen. ***PEB3*** eluted from this column at
around 450 mM Ka2so 4e Purified PEB3 (30 kDa) is shown
in Figure 2.

Determination of the isoelectric point (pI)
of the...For the PEB1
antigen, the pI- was found to be about 8 and for the
PEB3 antigen, the pI was greater than 9
The purified proteins were prepared for
amino acid...

...basic amino acids as
shown below in Table 1.

Table 1
AMINO ACID COMPOSITION OF
PEB ! AND ***PEB3*** ANTIGENS OF C. leluni
Amino Acid Mole %
PEB 1 PEB 3
Polar

Lysine 304,2 22e9
Histadine 0*6 le4
Arginine 2*0 4*0...

...03RPTH program. 30 of the
first 31 amino acid residues of the amino terminus of
PEB 1 and the 34 amino-terminus acids of PEB 3 were
defined as shown in Table 2.

Table 2
AMINO TERMINAL SEQUENCE OF
PEBI AND ***PEB3*** FROM C. JEJUNI 81@176
5 10 15
PEB1 Gly Glu Gly Lys Leu Glu Ser Ile Lys Ser Lys Gly Gln Leu Ile
PEB3 Asp Val Asn Leu Tyr Gly Pro Gly Gly Pro His Thr Ala Leu Lys...

...Val GlY Val LYS Asn Asp Val Pro His Tyr Ala Leu - Asp Gln Ala
PEB3 Asp Ile Ala Ser Lys Tyr Ser Glu Lys Thr Gly Val Lys Val Asn...
involve the
presence of fluorescein, an enzyme or a substrate
inside a liposome onto whose ***surface*** C. Jejuni antigens
are expressed. These liposomes are incubated with a
diluted body fluid sample to be tested,, and are
thoroughly washed, Any liposomes with immunoglobulins
on their surface forming an antigen/antibody complex
may be recognized by attaching a second antibody,
specific to the anti'genicity of the PEB1 and
PEB3 proteins for infected humans using an ELISA
The purified PEB1 and PEB3 proteins were
compared with A crude acid@extracted mixture of
C. jejuni proteins as antigens...

...described below.
- To sensitize ELISA plates (Nunc, inco,
Naperville, IL), purified proteins and the crude
surface protein preparation were diluted in 0.015 M
carbonate buffer, pH 9 One hundred ul...

...is shown below in Table 3.

Table 3. Seroconversion a to
C. jejuni proteins of ***Campylobacter*** -infected
nei'sons and'versons with other diarrheal diseases
b C -A e f
Patient isolate AE PEB1 PEB3 PEB4
Cw=lobacter ieluni/coli
Co coli + + +
F."coli + + + +
C. leiuni @g + +
coli @g @g...five strains of C. Jejuni, 15 of
,C, coli, 10 of C. fetus, 5 of ***Campylobacter*** laridis and
5 of Helicobacter r)ylori (formerly known as C. pylori)
were used in...defined as positive. In this system normal
rabbit serum did not'recognize any of the Campylobacter
strains, as expected (Table 4). In contrast, the
antisera to the mixture of acid@extracted...

...35 C. jejuni
strains, and all 15 C. coli strains, but none of the
other Campylobacter or Helicobacter isolates (Table 4
and Figure 3). Thus, the antisera to the PEB1 protein...

...both 100% sensitivity and specificity for C.

ieiuni and C. coli.

Table 4
RECOGNITION OF CAMPYLOBACTER AND HELICOBACTER CELLS
IN AN ELISA BY ANTISERA TO C. JEJUNI PROTEINS
% of Bacterial Strains...

...subsequently
dabsorbed with E. coli bacterial cells
All baterial iUrains used were clinical
isolates of Campylobacter and Helicobacter
species that had been stored at @700C prior
to subculture and testing.
EXAMPLE...

...in whole
bacterial cells by Western blot
To further confirm the specificity of
recognition of Campylobacter strains in ELISA by
antiserum to PEB1 antigen in Example 2, we performed
Western blotting...

...for the bands recognized by
this serum in preparations of whole bacterial cells of
various ***Campylobacter*** and Eelicobacter species. Whole
bacterial cells were prepared as described in
Example 2. 0.5...resolve is whether
PEBI antigen is a protein, To answer this, 24@hour
cultures of Campylobacter strains on blood agar plates
were harvested in sterile distilled H 20 (5 ml/plate...Figure 3), and had
a calculated
molecular weight of 28.9 kDa, indicating that the ***PEB*** !
antigen is a monomer. To compare effect of conditions
for extraction on polymerization of PEB1...
...poultry
We have considered the potential application
of the use of the PEB1 and/or PEB3 antigens in the
development of a vaccine against g.. Jejuni and C. coli
infections. To...

...of gastric acid and
proteolytic enzymes on the vaccine preparation,-the
whole PEB1 and/or PEB3 antigen (or a fragment of one or
both of these proteins) will be packaged either...0 mg of the antigens of
the invention, which may be either pure PEB1. pure
PEB3 , or a mixturi of PEB1 and ***PEB3*** . for example, a
dosage of about 10.0 mg of pure or mixed antigen.

To enhance delivery of PEB1 and/or PEB3 to
the gastrointestinal immune system the protein(s) [or a
fragment(s) of the proteins...

...limited, in preventing cholera.

For C. Jejuni, a parenteral vaccine could
include PEB1 and/or ***PEB3*** or fragments thereof. The
protein(s) or fragment(s) could be administered with an
adjuvant...

...to humans as
1.0 mg (range 0.5@5.0 mg) of antigen (PEB1, ***PEB3*** , or
mixture of both) in 1 ml of phosphate buffered saline
(pH7.4). With a were coated prior to use with PEB1 or ***PEB3***
antigens,

and ELISA materials for enzyme detection consisting of peroxidase@labeled goat anti@human 1gG...

...contains peroxidase-labelled goat or rabbit anti-human immunoglobulin and a source of PEB1 or ***PEB3*** antigens.

Another C., jejuni/g.* coli@specific test kit for detecting antibodies using the indirect immunofluorescence assay may include a compartmental container with PEB1 or PEB3 antigens, human test serum, phosphate buffered saline and fluorescein@conjugated goat anti@human IgG.

Finally...
...serum, fluorescent marker@ (or enzyme@ or substrate@) filled liposome with C. vylori antigens on their ***surface*** , and a ***surface*** @active agent. In this assay the container mght be a precoated tube or well with...

...the latex agglutination assay may include a compartmental container, hyperimmune serum to PEB1 and/or PEB3 conjugated to latex beads, and phosphate buffered saline or water.

The kits described above could...

...or C. coli organisms in fecal or water specimens, fecal or water specimens enriched for Campylobacteriby selective enrichment methods, or in colonies on solid media suspected as being Jee * or C...

Claim

1. An antigenic composition comprising a PEB1 antigen which, when obtained from Campylobacter jejuni without alteration of its natural composition, has an apparent molecular weight of 28 kDa...

...antigen being present in said antigenic composition at a concentration, relative to other acid extractable surface structures of CMylobacter jejuni, higher than that resulting from acid extraction of surface structures from whole cell ***Campylobacter*** leiuni.

2 The antigenic composition of claim 1, wherein said PEB1 antigen is present at a concentration, relative to other acid extractable porti,ons of Campylobacter,jejuni, of at least two times the natural concentration of said antigen resulting from acid...

...times the natural concentration of said antigen resulting from acid extraction.

4 A vaccine against Campylobacter jejuni and CgMylobacter coli infection, said vaccine comprising an amount of a PEB1 antigen effective...

...PEB1 antigen is present in said vaccine at a concentration, relative to other acid extractable surface structures, higher than that resulting from acid extraction of surface structures from whole cell

Campylobacter Jeluni,
S. The vaccine of claim 4 further
comprising a pharmaceutically acceptable adjuvant or
carrier...

...into biodegradable microspheres.

8 The vaccine of claim 4, wherein said
vaccine further comprises a PEB3 antigen which,, when
obtained from Cam-Pylobacter Jejuni without alteration
of its natural composition, has...
...kit comprising:
W- an immobilized antigenic
composition comprising a PEB1 antigen which, when
obtained from Campylobacter Jejuni without alteration
of its natural composition, has an apparent molecular
weight of about 28...

...in said antigenic composition at a
concentration higher than that resulting from acid
extraction 'of surface antigens from whole cell
Campylobacter lejuni;
W. means ...to said immobilized antigenic
composition.

10 A method for detecting the presence of
antibodies to Campylobacter jejuni or Campylobacter
Cali comprising contacting a test sample suspected of
including said antibodies with an amount of...

...pr6determined
positive threshold amount;
said antigenic composition comprising a
PEB1 antigen which, when obtained from Campylobacter
ieluni without alteration of its natural composition,
has an apparent molecular weight of about 28...

...antigen being
present in said antigenic composition at a
concentration, relative to other acid@extractable
surface structures of Camovlobacter jejuni, higher than
that resulting from acid extraction of surface
structures from whole cell Campylobacter jejuni,

11 The method of claim 10, wherein said
antigenic composition further comprises a PEB3 antigen
which, when obtained from Campylobacter jejuni without
alteration of its natural composition, has an apparent
molecular weight of about 30...

...antigen being present in said antigenic
composition at a concentration, relative to other acid
extractable surface structures of Campylobacter jejuni,
higher than that resulting from acid@extraction of
surface structures from whole cell Campylobacter
jejuni4 or Campylobacter coli,

12 The method of claim 10 wherein said
test sample is urine.

13 The...

...and
said antibodies are IgM.

16* A method of inducing production of protective antibodies against Campylobacter jejuni or Cam-pylobacter coli by animals, including humans, said method comprising the step of...

...of a vaccine comprising an effective amount of a PEB1 antigen which, when obtained from. ***Campylobacter*** iejuni without alteration of its natural composition, has an apparent molecular weight of about 28...with an antibody@containing composition for a time sufficient to allow said antibodies to bind Campylobacter jejuni or Campylobacter coli, if present in said sample to form an organism/antibody complex, and then determining...

...chromatography under native conditions), and an isoelectric point of

23 An antigenic composition comprising a PEB3 antigen which, when obtained from Campylobacter ieluni without alteration of its natural composition, has an apparent molecular weight of 30 kDa...

...dodecyl sulfate polyacrylamide gel under reducing conditions), and an isoelectric point greater than 9 said PEB3 antigen being present in said antigenic composition at a concentration, relative to other acid extractable surface structures of Camr)vlobacter lejuni, higher than that resulting from 6d extraction of surface structures from whole cell aci Cammylobacter leluni.

24 The antigenic composition of claim 23, wherein said PEB3 antigen is present at a concentration, relative to other acid extractable portions of Campylobacter ieluni, of at least two times the natural concentration of said antigen resulting from acid extraction,

25 The antigenic composition of claim 23, wherein said PEB3 antigen is present at a concentration, relative to other acid extractable portions of Camipylobacter Jejuni...

...times the natural concentration of said'antigen resulting from acid extraction.

26 A vaccine against Campylobacter Jelouni and Campylobacter coli infection, said vaccine comprising an amount of a PEB3 antigen effective to induce production of protective antibodies against Camvylobacter leluni or Campylobacter coll by animals, including humans, who have be treated with said vaccine, said PEB3 antigen having, when obtained from Campylobacter Jejuni without a change in its composition, an apparent molecular weight of about 30 kDa isoelectric point greater than 9

27 The vaccine of claim 26 wherein said PEB3 antigen is present in said vaccine at a concentration, relative to other acid extractable surface structures, higher than that resulting from acid extraction of surface structures from whole cell

Camipylobacter Jejuni.

28 The vaccine of claim 26 further comprising a pharmaceutically acceptable adjuvant or carrier-.

29 The vaccine of Claim 26, wherein said PEB3 antigen is incorporated, for purposes of oral ingestion, into biodegradable microspheres.

30* The vaccine of claim 26, wherein said vaccine further comprises aPEB1 antigen which, when obtained from Campylobacter jejuni without alteration of its natural composition, has an apparent molecular weight of 28 kDa...

...point of about 8

31 A diagnostic test kit for detecting Camr)ylobacter lejuni or Campylobacter coli infection, said test kit comprising:
(a) an immobilized antigenic composition comprising a PEB3 antigen which, when obtained from Cam-Pylobacter jejuni without alteration of its natural composition, has...

...sulfate polyacrylamide gel under reducing conditions), and an isoelectric point greater than 9.3, said ***surface*** antigen being present in said-antigenic composition at a concentration higher than that resulting from acid extraction of surface antigens from whole cell Campylobacter lejuni;
(b) means for passing-bodily fluid taken from an animal to be tested over...

...32* A method for detecting the presence of antibodies to C M ylobacter jejuni or Campylobacter coli comprising contacting a test sample suspected of including said antibodies with an amount of...

...said antigen/antibody complex exceeds a predetermined positive threshold amount;
said antigenic composition comprising a PEB3 antigen which, when obtained from,Campylobacter leiuni without alteration of its natural composition, has an apparent molecular weight of 30 kDa...

...dodecyl sulfate polyacrylamide gel under reducing conditions), and an isoelectric point greater than 9 said PEB3 antigen being present in said antigenic composition at a concentration, relative to other acid-extractable surface structures of Campyl-obacter lejuni, higher than that resulting from acid extraction of surface structures from whole cell Cam,pylobacter leiuni,

33 The method of claim 32, wherein said antigenic composition further comprises a PEBI antigen which, when obtained from Campylobacter jejuni without alteration of its natural composition, has an apparent molecular weight of 28 kDa...antigen being present in said antigenic composition at a concentration, relative to other acid extractable surface structures of Campylobacter jejuni, higher than that resulting from acid@extraction of

surface structures from whole@cell Campylobacter ieiuni.

34 The method-of claim 32 wherein said test sample is urine.

35 The...

...said antibodies are Igm*
38* A method of inducing production of protective antib'odies against Campylobacter jejuni or Cam-pylobacter coli by animals, including humans, said method comprising the step of...

...infection is desired, an effective amount of a vaccine comprising an effective amount of a PEB3 antigen which, when obtained from'Campylobacter ieiuni without alteration of its natural composition, has an apparent molecular weight of 30 kDa...

...38, wherein said vaccine is clinically administered to a human,

43 Antisera raised against a PEB3 antigen which, when obtained from CAMylobacter jejuni without alteration of its natural composition, has an...

...point greater than 9

44 A method of determining the presence of Cam2ylobacter jejuni or Campylobacter coli in a test sample comprising the steps of contacting said test sample with an...

...predetermined positive threshold value; said antibody-containing composition comprising immunoglobulin from antisera raised against a PEB3 antigen which, when obtained from Campylobacter ieluni without alteration of its natural composition, has an apparent molecular weight of 30 kDa...

? logoff

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      01aug07 13:26:33 User228206 Session D2713.4
           $3.09    0.910 DialUnits File155
              $0.00  76 Type(s) in Format  6
              $9.68  44 Type(s) in Format  9
           $9.68  120 Types
     $12.77  Estimated cost File155
           $3.26    0.658 DialUnits File144
              $0.00  25 Type(s) in Format  6
           $0.00  25 Types
      $3.26  Estimated cost File144
           $4.92    0.819 DialUnits File5
              $2.30  1 Type(s) in Format  3
              $0.00  27 Type(s) in Format  6
           $2.30  28 Types
      $7.22  Estimated cost File5
           $7.41    0.623 DialUnits File73
              $0.00  8 Type(s) in Format  6
           $0.00  8 Types
      $7.41  Estimated cost File73
           $0.91    0.155 DialUnits File156
      $0.91  Estimated cost File156
           $1.18    0.287 DialUnits File65
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WEST Search History

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END OF SEARCH HISTORY

Search in UniProt Knowledgebase (Swiss-Prot and TrEMBL) for: peb3 campylobacter

UniProtKB/Swiss-Prot Release 54.0 of 24-Jul-2007
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- Number of sequences found in UniProt Knowledgebase (Swiss-Prot$_{(0)}$ and TrEMBL)$_{(9)}$: **9**
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Search in UniProtKB/Swiss-Prot: There are matches to 0 out of 276256 entries

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Search in UniProtKB/TrEMBL: There are matches to 9 out of 4672908 entries

A1VY12_CAMJJ
Major antigenic peptide PEB3 {GENE:OrderedLocusNames=CJJ81176_0315} - Campylobacter jejuni subsp. jejuni serotype O:23/36 (strain 81-176)

A3YJG7_CAMJE
Major antigenic peptide PEB3 {GENE:ORFNames=CJJCF936_0312} - Campylobacter jejuni subsp. jejuni CF93-6

A3YQL7_CAMJE
Major antigenic peptide PEB3 {GENE:ORFNames=CJJ26094_0304} - Campylobacter jejuni subsp. jejuni 260.94

A3ZJJ7_CAMJE
Major antigenic peptide PEB3 {GENE:ORFNames=CJJ8425_0313} - Campylobacter jejuni subsp. jejuni 84-25

A5KFD0_CAMJE
Major antigenic peptide PEB3\cell binding factor 2 {GENE:ORFNames=Cj8486_0604} - Campylobacter jejuni subsp. jejuni CG8486

A5KIA8_CAMJE
Major antigenic peptide PEB3 {GENE:ORFNames=Cj8486_0277c} - Campylobacter jejuni subsp. jejuni CG8486

Q0PBL7_CAMJE
Major antigenic peptide PEB3 precursor {GENE:Name=peb3;

OrderedLocusNames=Cj0289c} - Campylobacter jejuni

Q5HWH8_CAMJR

Major antigenic peptide PEB3 {GENE:OrderedLocusNames=CJE0337} - Campylobacter jejuni (strain RM1221)

Q9R5U0_CAMJE

PEB3=MAJOR antigenic peptide (Fragment) - Campylobacter jejuni

Bacon, D. J., C. M. Szymanski, et al. (2001). "A phase-variable capsule is involved in virulence of **Camp** jejuni 81-176." Mol Microbiol **40**(3): 769-77.

Campylobacter jejuni strain 81-176 (HS36, 23) synthesizes two distinct glycan structures, as visu immunoblotting of proteinase K- digested whole-cell preparations. A site-specific insertional muta kpsM gene results in loss of expression of a high-molecular-weight (HMW) glycan (apparent Mr 2 85 kDa) and increased resolution of a second ladder-like glycan (apparent Mr 26-50 kDa). The kps 81-176 is no longer typeable in either HS23 or HS36 antisera, indicating that the HMW glycan stru serodeterminant of HS23 and HS36. Both the kpsM-dependent HMW glycan and the kpsM-indepe like structure appear to be capsular in nature, as both are attached to phospholipid rather than lipid Additionally, the 81-176 kpsM gene can complement a deletion in Escherichia coli kpsM, allowing expression of an alpha2,8 polysialic acid capsule in E. coli. Loss of the HMW glycan in 81-176 kp increases the surface hydrophobicity and serum sensitivity of the bacterium. The kpsM mutant is a significantly reduced in invasion of INT407 cells and reduced in virulence in a ferret diarrhoeal dis The expression of the kpsM-dependent capsule undergoes phase variation at a high frequency.

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LPS analysis

LPS was isolated by a modification of the method of Hitchcock and Brown (1983). Bacteria from 2-day-old blood agar plates were directly solubilized in 100 μl of lysing buffer containing 31.25 mM Tris-HCl, pH 6.8, 4% SDS, 0.025% bromophenol blue and 20% glycerol. Samples were heated at 100°C for 10 min and centrifuged at 13 000 r.p.m. for a further 10 min. Aliquots (20 μl) of the supernatant were removed to a fresh tube, to which 1 μl of a 20 mg ml^{-1} solution of proteinase K (Sigma) was added, and the mixture was incubated at 65°C for 2 h. Before electrophoresis, proteinase K-treated samples were boiled and centrifuged as above.

Proteinase K-treated LPS samples were fractionated using SDS–PAGE with a Hoefer minigel system. For increased resolution of low-molecular-weight components, the tricine-SDS–PAGE system of Schägger and von Jagow (1987) was used. After electrophoresis on 12.5% acrylamide gels, LPS was visualized by a carbohydrate-specific silver staining method (Tsai and Frasch, 1982) or, alternatively, gels were Western blotted onto polyvinylidene difluoride (PVDF) membrane (Millipore) using a semi-dry electroblotting apparatus (Hoefer). Blots were blocked overnight at 4°C in Tris-buffered saline containing 0.01% Tween 20 (TBST) and 3% skimmed milk. After blocking, blots were incubated for 1 h with Penner antiserum at a dilution of 1:100 in TBST containing 1% bovine serum albumin (TBST-BSA), washed three times in TBST, followed by incubation with peroxidase-labelled anti-rabbit IgG (Sigma) diluted 1:1000 in TBST-BSA for a further hour. After washing as above, blots were developed using the DAB staining kit with nickel enhancement according to the manufacturer's instructions (Vector Laboratories).

Treatment with phospholipases

The phospholipases used in this study were purchased from Sigma: phospholipase C type IX from *Bacillus cereus*; phospholipase A2 from bee venom; and phospholipase D type I from cabbage. One loop of bacteria from 2-day-old blood agar plates was resuspended in 0.5 ml of saline and incubated at 100°C for 0.5 h. The samples were centrifuged, supernatants were adjusted to 50 mM Tris-HCl, pH 8.0, and, after the addition of one unit of each phospholipase, incubated for 1 h at 30°C followed by incubation at 37°C for 12–14 h. The samples were analysed using Western blotting as described above.

Hydrophobic interaction chromatography

The surface hydrophobicity of wild-type and mutant strains was examined using a modification of the method of Field *et al.* (1993). Disposable plastic columns were packed with Octyl Sepharose CL-4B to a height of 2 cm and washed with 10 ml of 0.2 M ammonium sulphate in 10 mM sodium phosphate buffer, pH 6.8 (buffer A). Cells were harvested from 2-day-old blood agar plates, resuspended in PBS, and a 100 μl aliquot was gently pipetted onto the surface of the column or, as a control, into 5 ml of buffer A. Columns were then washed with 5 ml of buffer A, and the absorbance values, at a wavelength of 600 nm, of both the column flowthrough and control samples were determined. Results were expressed as a percentage of cells adsorbed to the column. All experiments were performed in triplicate and a mean value and standard error calculated. Student's *t*-test was used to determine significance expressed as a *P*-value.

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REFERENCE PMID:11278778
AUTHORS Cipollo JF, Trimble RB, Chi JH, Yan Q, Dean N.
TITLE The yeast ALG11 gene specifies addition of the terminal alpha 1,2-Man to the Man5GlcNAc2-PP-dolichol N-glycosylation intermediate formed on the cytosolic side of the endoplasmic reticulum.
JOURNAL J Biol Chem 276:21828-40 (2001)

Viable counts were determined by serial dilution onto MH agar. Ferrets were anaesthetized with acepromazine–ketamine intramuscularly and fed 10.0 ml of bacterial culture via a paediatric intubation tube. At 1 h after challenge, 2.8 ml kg^{-1} tincture of opium was administered intraperitoneally to reduce peristalsis. After infection, animals were monitored three times daily for signs of diarrhoea.

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is an important first step in identifying further genes involved in the generation of this intriguing polysaccharide and in understanding its biological significance.

Taken together, these data demonstrate the efficiency of the *C. jejuni* sequencing data for the rapid identification of previously unsuspected virulence determinants.

Experimental procedures

Bacterial strains and growth conditions

C. jejuni strains (Table 1) were grown at 37°C on 7% blood agar or Mueller–Hinton agar plates in a microaerobic atmosphere (CampyGen; Oxoid) for 2 days. The *E. coli* XL2 Blue MRF′ (Stratagene) strain, used in cloning experiments, was grown overnight at 37°C on LB agar plates. For swarming experiments, 0.3% Mueller–Hinton agar plates were used. Where necessary, the agar plates were supplemented with the antibiotics kanamycin, at a concentration of 20 or 50 μg ml^{-1} for *E. coli* and *C. jejuni* respectively, and/or ampicillin, at a concentration of 100 μg ml^{-1} for *E. coli*.

Serotyping

Antigenic extracts of *C. jejuni* strains were prepared in the UK laboratory as has been described previously (Lastovica *et al.*, 1986), and sent under code to the Cape Town laboratory for serotyping. Serotyping was performed with antisera to the complete set of 67 reference strains of the established *Campylobacter* serotyping scheme based on thermostable antigens (Penner *et al.*, 1983).

Cloning and sequencing of kpsM and kpsT genes

Chromosomal DNA was extracted by lysis with 1% SDS in the presence of 100 μg ml^{-1} proteinase K, followed by phenol–chloroform extraction and precipitation by isopropanol. DNA was resuspended in TE buffer to a final concentration of 100 μg ml^{-1} and stored at 4°C. Construction of the random 2 kb library has been described elsewhere (Karlyshev *et al.*, 1999). DNA sequencing was performed on an ABI 373 automatic sequencer using an ABI PRISM™ Dye Terminator Cycle Sequencing Kit (Perkin-Elmer). The insert in plasmid pA4 containing a full-length *kpsM* gene and a fragment of the *kpsT* gene was labelled by incorporation of digoxigenin-dUTP (Boehringer Mannheim) in a PCR with vector-specific primers and hybridized to a previously described cosmid library of *C. jejuni* NCTC 11168 (Karlyshev *et al.*, 1998). Cosmids extracted from the positive colonies were used to extend the sequence of the pA4 plasmid insert by primer walking (GenBank accession number AJ000856).

Sequence analysis

Multiple alignments were generated using the CLUSTAL W (GCG) package (Thompson *et al.*, 1994). Similarity searches were carried out using the NCBI (National Centre for Biotechnology Information, Bethesda, MD, USA) BLAST programs (http://www.ncbi.nlm.nih.gov/BLAST). Program PSORT (http://psort.nibb.ac.jp/form.html) was used to predict the cellular location of proteins. Potential transmembrane regions were determined using the TMPRED program (http://ulrec3.unil.ch/software/TMPRED_form.html), and hydrophobicity profiles were generated using the Kyte and Doolittle algorithm (Kyte and Doolittle, 1982) in GENEJOCKEY II. The SCNPROSITE program (http://expasy.hcuge.ch/sprot/scnpsit1.html) was used to detect the A-region of the ATP-binding motif, whereas the B-region was found by comparison with published data (Fath and Kolter, 1993).

Plasmid construction

A kanamycin resistance (*kn*^{r}) cassette (Trieu-Cuot *et al.*, 1985) was used for making constructions suitable for site-directed inactivation of the *kpsM*, *kpsS* and *kpsC* genes via allelic replacement. The *kpsM* gene in plasmid pA4 was disrupted by the insertion of the *kn*^{r} cassette into a unique *Nhe*I restriction site located at nucleotide 517 from the start codon of the 783 nucleotide gene. Briefly, *Nhe*I-digested plasmid pA4 and a *Bam*HI fragment of plasmid pGMK30 containing the *kn*^{r} cassette were blunt ended, ligated, transformed into *E. coli* and kanamycin-resistant colonies were selected. A plasmid with the *kn*^{r} cassette inserted in the same orientation as the *kpsM* gene was identified using PCR with vector-derived primer AK3 (GTAAAACGACGGCCAGTG) and *kn*^{r} cassette-derived reverse primer DL4 (TGTTGCTGTCTCCCAGGTCG). Fragments containing *kpsC* and *kpsS* genes were PCR amplified using the AK104 (GGTTGGGGACGCAA AAAATCAGGT)/AK105 (GCACCTGCTACAAGCTCTTCTAGGCT) and AK106 (GGTAAAAATGTTTTACTCTTGC AAGGGCCT)/AK107 (GGTTTATAAGCATGAGCTTCAC GCCA) pairs of primers respectively. After cloning of the PCR products into vector pGEM-T-Easy (Promega), a blunt-ended *Bam*HI fragment containing the *kn*^{r} cassette was inserted in the *Swa*I sites at positions 617 nucleotides (*kpsC*) and 477 nucleotides (*kpsS*) from the translation start codon. Insertion into the *kpsC* gene also generated a 276 nucleotide deletion as a result of the presence of a second *Swa*I site in the gene. After transformation, the derivatives with direct orientations of the *kn*^{r} cassette were selected using PCR with a combination of the cassette-derived primer DL3 (ACCCAGCGAACCATTTGAGG) and the corresponding target-derived primer (Fig. 4).

Insertional inactivation of C. jejuni kpsM, kpsC and kpsS genes

Plasmids with the fragments of the *kpsM*, *kpsC* and *kpsS* genes containing an inserted *kn*^{r} cassette were used to transform *C. jejuni* cells by either electroporation or natural transformation using 1–5 μg of DNA (Wassenaar *et al.*, 1993). In order to identify allelic replacement mutants, primers AK55/AK59 (*kpsM*), AK104/AK105 (*kpsC*) and AK106/AK107 (*kpsS*) were used to PCR amplify regions flanking *kn*^{r} cassette insertion sites with the cell lysates prepared as described previously (Karlyshev and MacIntyre, 1996).

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CAS Registry Number: 0 (Amino Acids); 0 (Bacterial Proteins); 0 (Membrane Glycoproteins); 0 (surface array protein, bacteria)
Record Date Created: 19880606
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6/9/35 (Item 35 from file: 155)
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07402962 PMID: 3298060
Two-dimensional gel electrophoresis and immunoblotting of ***Campylobacter*** outer membrane proteins.
Dunn B E; Blaser M J; Snyder E L
Infection and immunity (UNITED STATES) Jul 1987, 55 (7) p1564-72, ISSN 0019-9567--Print Journal Code: 0246127
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We characterized outer membrane proteins (OMPs) from selected ***Campylobacter*** jejuni, C. coli, and C. fetus strains by two-dimensional gel electrophoresis (2DGE), using isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and by immunoblotting with immune rabbit serum. The flagellar band with a molecular mass of 63 kilodaltons (kDa) demonstrated previously by one-dimensional SDS-PAGE was shown by 2DGE to consist of one or several charge trains, depending upon the species, strain, and type of preparation studied; each of the individual peptides was found to be antigenic by immunoblotting. In contrast, in all of the strains studied, the major OMP (43 to 44 kDa) of C.jejuni and C. coli consisted of a single isomeric form which was weakly immunogenic. Several minor proteins (29 to 31 kDa) were found to be strongly immunogenic by immunoblotting. C. fetus strains possessed two major OMPs of 45 to 47 kDa, each of which consisted of either a single isomer or a major isomer comprising at least 90% of the major OMP. Serum-resistant strains of C. fetus possessed an acid-labile 100-kDa glycoprotein (pI, 4.1) which was markedly diminished or absent in serum-sensitive strains. These 2DGE analyses provide information that is useful in taxonomic and epidemiologic studies and for the purification of surface antigens for the development of campylobacter vaccines and may also facilitate the identification of specific virulence factors.
Descriptors: *Bacterial Outer Membrane Proteins--analysis--AN; *Campylobacter--analysis--AN; Electrophoresis, Polyacrylamide Gel; Immunosorbent Techniques; Isoelectric Point; Molecular Weight; Sonication; Species Specificity
CAS Registry Number: 0 (Bacterial Outer Membrane Proteins)
Record Date Created: 19870729
Record Date Completed: 19870729

6/9/36 (Item 36 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07354875 PMID: 3552997
Conservation and diversity of Campylobacter pyloridis major antigens.
Perez-Perez G I; Blaser M J
Infection and immunity (UNITED STATES) May 1987, 55 (5) p1256-63, ISSN 0019-9567--Print Journal Code: 0246127

and mutant strains. Here we report that a cloned silent gene (sapA1) in C. fetus can express a functional full-length S-layer protein in Escherichia coli. Analysis of sapA and sapA1 and partial analysis of sapA2 indicate that a block of approximately 600 bp beginning upstream and continuing into the open reading frames is completely conserved, and then the sequences diverge completely, but immediately downstream of each gene is another conserved 50-bp sequence. Conservation of sapA1 among strains, the presence of a putative Chi (RecBCD recognition) site upstream of sapA, sapA1, and sapA2, and the sequence identities of the sapA genes suggest a system for homologous recombination. Comparison of the wild-type strain (23D) with a phenotypic variant (23D-11) indicates that variation is associated with removal of the divergent region of sapA from the expression locus and exchange with a corresponding region from a sapA homolog. We propose that site-specific reciprocal recombination between sapA homologs leads to expression of divergent S-layer proteins as one of the mechanisms that C. fetus uses for antigenic variation.

Descriptors: *Antigens, Bacterial--genetics--GE; *Bacterial Proteins --genetics--GE; *Campylobacter fetus--genetics--GE; *Genes, Bacterial ; *Membrane Glycoproteins; Base Sequence; Cloning, Molecular; DNA, Bacterial--genetics--GE; Gene Rearrangement; Molecular Sequence Data; Phenotype; Recombination, Genetic; Restriction Mapping; Sequence Alignment; Sequence Homology, Nucleic Acid; Species Specificity

Molecular Sequence Databank Number: GENBANK/L15800

CAS Registry Number: 0 (Antigens, Bacterial); 0 (Bacterial Proteins); 0 (DNA, Bacterial); 0 (Membrane Glycoproteins); 0 (surface array protein, bacteria)

Gene Symbol: sapA; sapA1; sapA2; sapA3

Record Date Created: 19930907

Record Date Completed: 19930907

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DIALOG(R)File 155:MEDLINE(R)

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Isolation and characterization of two Campylobacter glycine-extracted proteins that bind to HeLa cell membranes.

Kervella M; Pages J M; Pei Z; Grollier G; Blaser M J; Fauchere J L

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Two immunogenic proteins of 27 (CBF1) and 29 (CBF2) kDa from enteropathogenic Campylobacter species appear to bind to mammalian cells. We purified these two proteins from a pathogenic and adherent Campylobacter jejuni strain to homogeneity by using acid extraction, preparative gel electrophoresis, and electroelution. Polyclonal rabbit antisera to these proteins were prepared. Immunologic studies indicate that CBF1 corresponds to the PEB1 and CBF2 corresponds to the PEB4 described by Pei et al. (Z. Pei, R. T. Ellison, and M. Blaser, J. Biol. Chemical 226:16363-16369, 1991). Immunogold labeling of a C. jejuni adherent strain with anti-CBF1, anti-CBF2, and anti-PEB1 suggested that CBF1 (PEB1) is ***surface*** exposed while CBF2 (PEB4) is not. Analysis of whole-cell extracts from 14 strains by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis with 7 M urea and immunoblotting with antisera to CBF1 and CBF2 suggests that CBF proteins from adherent and nonadherent strains are

different. Use of purified proteins in a microassay of adherence to cellular membranes indicated that CBF1 was much more adherent than CBF2. Adherence of C. jejuni to viable HeLa cells was markedly reduced with the antiserum to CBF1, whereas the CBF2 antiserum was a poor inhibitor. Purified CBF1 competitively inhibited adherence of whole bacteria to HeLa cells, whereas purified CBF2 was no better a competitor than bovine serum albumin. Adherence of CBF2 was markedly reduced in the presence of Tween 20 or SDS, whereas adherence of CBF1 was reduced only by SDS. We conclude that (i) CBF1 (PEB1) is ***surface*** exposed and may be the key protein for C. jejuni adhesion and (ii) CBF2 (PEB4) may be complexed with CBF1 and may passively coadhere with CBF1 under certain experimental conditions. Adherent and nonadherent strains contain different isotypes of these two proteins which could be useful markers of C. jejuni adhesion.

Descriptors: *Bacterial Adhesion; *Bacterial Proteins --isolation and purification--IP; *Campylobacter--chemistry--CH; Antibody Specificity ; Bacterial Adhesion--drug effects--DE; Bacterial Proteins--metabolism--ME; Bacterial Proteins--pharmacology--PD; Epithelium--metabolism--ME; Glycine; Hela Cells; Humans; Immune Sera--immunology--IM

CAS Registry Number: 0 (Bacterial Proteins); 0 (Immune Sera); 56-40-6 (Glycine)

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Pathogenesis of Campylobacter fetus infections: critical role of high-molecular-weight S-layer proteins in virulence.

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Wild-type Campylobacter fetus strains possess high-molecular-weight S-layer proteins (S+) and are highly resistant to serum-mediated killing and phagocytosis. Spontaneous mutant strains lacking these proteins (S-) are serum and phagocytosis sensitive and have reduced virulence in a mouse model. Intact S+ cells were treated with pronase, which made them S- although genotypically S+ and had essentially no effect on other cellular proteins or on viability. Treatment with pronase, but not buffer alone, rendered these cells serum and phagocytosis sensitive and reduced mouse virulence to the level observed for the S- mutant cells. In related studies, purified S-layer proteins diminished neutrophil chemoluminescent responses to a heterologous particulate antigen. Finally, passive administration of antiserum to the 97-kDa S-layer protein partially protected mice against lethal challenge with the S+ strain. These studies define the contribution of the S-layer proteins to C. fetus virulence.

Descriptors: *Bacterial Proteins--immunology--IM; *Campylobacter Infections--microbiology--MI; *Campylobacter fetus--pathogenicity --PY; *Membrane Glycoproteins; Animals; Antigens, Bacterial--physiology--PH ; Blood Bactericidal Activity; Campylobacter Infections--immunology --IM; Campylobacter fetus--immunology--IM; Campylobacter fetus --metabolism--ME; Chemiluminescent Measurements; Immunization, Passive;